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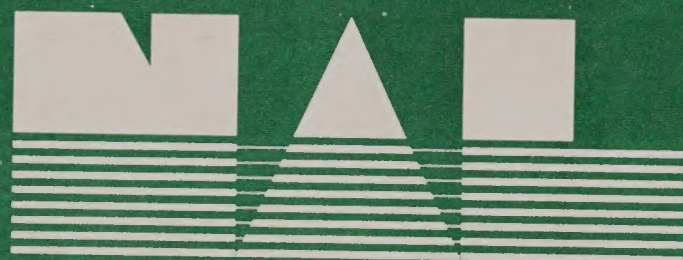
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**Progress Report**  
**on**  
**New Crops Research**

ARS New Crops Meeting  
Washington, D.C.  
January 25, 1963



**United States  
Department of  
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JUN 3 1997

CATALOGING PREP.

1/25/63

Dr. Thomas & Jones  
Dr. Hanson  
Poats

Opening Remarks - Dr. MacLay.

Has noted interest of trade groups in new oils.

2 Jones - Sample Procurement.

Greatest need for follow up work - own staff.

Had done pretty good job on calendula (Dimorphotera)

Have done pretty good job of sample prep this last summer on Linnanthus.

S somewhat less - Leaguesells - more work needed  
enrich - not encouraging  
in what was found.

Nemoria

A Taxonomist is taking this as a special assignment.

S Lamm screening on PL480 - Looks like program  
going well - Coop w General Mills. They examine

for colander. Points to some of the. May

have more promise than D isocorea. Stems in  
fruits and leaves. PL480 in Colombia, another to

be initiated in India.

Currently collections about in equilibrium with screening at NU.

MacLay believes should have a backlog. Colson can build up.



## GENERAL COMMENTS ON THE SCREENING PROGRAM

1. The seed screening program is progressing smoothly, with a good flow of samples, and a chemical survey phase limited only by manpower turnover and total personnel available for the job. In the screening aspect of the pulp fiber program the chemists require an increased amount of guidance from botanists to enable and facilitate selection of preferred samples that merit more detailed future research.
2. Selected results from the oilseed chemical screening and characterization investigations that may warrant calling to the group's attention are the following:

Cuphea - Two new species in this versatile genus contain seed oil mostly (70%) comprised of  $C_8$  saturated acid. By choice of species of Cuphea one can then grow oils predominating in  $C_8$ ,  $C_{10}$  or  $C_{12}$  acids.

Limnanthes - The novel composition originally deduced for a single species is general for the genus, with some variability for individual components as shown in more detail later in the report.

Thalictrum - Oils from two recently acquired species (minus, lucidum) contain 42 to 46% of the new triene acid reported last year, the trans-5,cis-9,cis-12-octadecatrienoic acid.

Crepis - A Turkish accession contains in its seed oil approximately 60% of a new acetylenic acid, characterized as cis-9-octadecen-12-ynoic acid.

Cis-11-eicosenoic acid - Three rich sources have been found, having the indicated percentages in their oils: Selenia (58), Marshallia (44), Alyssum (42).

Ipomoea - Twenty to 25% of seed lipids are glycosides of 11-hydroxypalmitic acid and a mixture of three deoxyhexoses in an average proportion of 2 sugar molecules per fatty acid molecule.

Onobrychis - Seed from a Turkish legume has 57% protein and 13% oil. Increased emphasis, on a worldwide basis, on utilization of vegetable protein sources may make this finding worth followup studies.

3. Further followup studies are indicated on several mucilage-bearing seed species, including Sphinctospermum constrictum and Cassia marilandica. The former has newly been found in screening to contain 24% of N-free polysaccharide; the latter has been found to be less toxic than some of the crotalaris previously considered, and should have some evaluation tests made on its mucilage as a paper additive.



## SAMPLE PROCUREMENT

During the past year sample procurement was accomplished largely through PL 480 projects. Turkey provided 120 samples, practically all seed, and another 150 samples are being shipped. Israel sent in 130 accessions, about equally divided between seed and whole-plant samples, and another 50 are on their way. Spain tripled their effort of the previous year and submitted 150 samples, about 1/4 of which were seed collections and 3/4 whole-plant collections. Yugoslavia, which did not get into field collecting last year, has recently shipped 166 samples from this past summer's collecting and has another 100 samples to ship. Pakistan got off to an excellent start this past spring, and we have already received 137 collections from them. Also, by special arrangement, they provided us with 178 pounds of Vernonia anthelmintica seed for EU's oil studies. The project in Uruguay seems to be having difficulties in comprehending the objectives of this type of collecting program. They have submitted 177 samples, but relatively few of these were large enough for chemical screening. A general collecting project has been successfully negotiated with Seoul University, South Korea, and we can look forward to receiving samples next summer from this floristically interesting area.

Of outstanding interest is the seed collection of Crepis foetida ssp. rhoeadifolia from Turkey. Professor Tosun, Principal Investigator of the Turkey project, reports that this is an annual and a heavy seeder. Our request for additional amounts of seed of this species was given prompt attention, and an additional 2 pounds of seed is being sent.

Field collecting by staff botanists was largely restricted to the Pacific Coast States during the spring and summer months. Intensive seed collecting and field study of the genus Limnanthes provided analytical and propagation samples of all species of the genus and ecological information pertinent to a more critical evaluation of crop potential of species of this genus. As a result of this field study we are much more optimistic that a new oilseed crop can be developed from elements of the genus Limnanthes.

Program limitations dictate that the above pattern, of general collecting being accomplished under PL 480 projects and special collecting by staff of the New Crops Research Branch, will continue for the foreseeable future. Intensive collecting and field study of Vernonia, section Stengelina, is being planned to provide the basis for a better evaluation of Vernonia anthelmintica, and its relatives, as a potential oilseed crop. A number of leguminous genera from northern and central Mexico (Cassia, Crotalaria, Sesbania, etc.) merit additional sampling and field study as potential sources of seed gums and pulp fibers. This is another activity planned for staff botanists as soon as funds permit.



FHEarle

## SEED SAMPLES RECEIVED AND ANALYZED

	<u>Increase since Dec. 1, 1961</u>	<u>Total to Oct. 31, 1962</u>
Seed Samples Received	803	4185
Families	4	153
Genera	139	1052
Species	431	2459
Incomplete identification	49	178
Seed Samples Analyzed	491	3642
>20% oil	242	1562
10-20% oil	106	658
>35% protein	48	489
>60% oil and protein	85	581
<35% oil and protein, no starch	83	663
Starch test positive	59	742
Oil Samples Analyzed		
Isomerization	0	187
GLC	214	716
Samples Disposed of Without Screening	15	65
Increase Samples from States or Regional Programs		
Received	107	314
Analyzed	16	136

A manuscript containing extensive tabular primary analytical data on the first 1400 seed samples has been accepted for publication in ECONOMIC BOTANY.



## SEED LIPID SCREENING AND CHARACTERIZATION RESEARCH

## I. Information on Assigned Oilseeds

A. Dimorphotheca and related genera. Sixteen samples of Calenduleae were analyzed. Most were so small in amount that examination was limited to a single analysis for percentage of oil, and ultraviolet analysis for conjugated acids. Dimorphecolic acid was found in the two samples of Castalis nudicaulis (35 and 48 percent), in Osteospermum fruticosum (61 percent) and in O. jucundum (71 percent). These samples contained 2 to 5 percent of conjugated triene acids. The remaining samples, Calendula arvensis and six species of Osteospermum, contained conjugated triene in amounts ranging from 11 to 42 percent and no dimorphecolic acid. Total oil content of these accessions was low, the highest (on an as-is basis) being 16.6 percent. (CPR ranges from fair to good.)

B. HBr-Reactive acids other than those of Calenduleae. (Method includes epoxy, cyclopropenoid, and dimorphecolic-type acids.) Titration with HBr on the oils obtained in screening showed 7 members of the mallow family with oils containing from 11 to 20 percent of HBr reactive components calculated as  $C_{18}$  epoxy acid. There were also two Umbelliferae, two Compositae and one Guttiferae (Hypericum perforatum, St. John's Wort). A total of 32 samples showed 5 percent or more HBr-reactive acid by titration. Those having 10 percent or more HBr-reactive acids are listed below:

			CPR
Althaea officinalis	Malvaceae	20	Good
Althaea setosa	Malvaceae	11	Good
Lavatera punctata	Malvaceae	12-15	Good
Malva aegyptia	Malvaceae	13	Fair to good
Malva sylvestris	Malvaceae	12	Good
Modiola caroliniana	Malvaceae	11	Fair
Polytaenia nuttallii	Umbelliferae	15	Good
Daucus maximus	Umbelliferae	13	Good
Lactuca scariola	Compositae	10	Fair
Tragopogon dubius	Compositae	10	Fair
Hypericum perforatum	Guttiferae	16	Poor to fair

C. Limnanthes. Eighteen samples of Limnanthes including seven species and four varieties have been analyzed. Oil content of the seed ranged from 20 to 33 percent, and the amounts of acids longer than  $C_{18}$  ranged from 95 to 98 percent. The data obtained are compiled in the following table.



Limnanthes Species	Oil Composition				Seed Oil Content %
	<C <sub>20</sub>	20:1	22:1	22:3	
alba	2.4	61.0	14.9	20.3	27
alba var. versicolor	1.8	59.7	27.9	10.1	31
bakeri	4.6	57.3	24.7	10.6	26
douglasii (4)	2.8-5.1	57.8-64.8	17.6-20.0	10.0-15.2	24-30
douglasii var. nivea (4)	1.9-4.3	59.7-66.7	18.4-23.3	2.8-10.7	26-33
douglasii var. rosea (2)	1.5,3.0	71.7,76.8	7.5,11.6	10.3,12.0	20-22
floccosa	2.5	58.6	23.5	12.0	28
gracilis	2.3	55.1	28.9	12.6	29
gracilis var. parishii	3.6	59.3	23.5	12.2	33
montana	2.1	51.6	25.0	16.6	26
striata	4.6	64.7	13.6	16.0	29

Relative Qualifications of Varieties as Seed Crop Prospects.

Species or Variety	Habit	H <sub>2</sub> O Requirement	Yield	Seed Retention	% Oil
gracilis	good	medium	good	fair	28.8
alba	good	light	good	excellent	26.8
nivea (Willets)	good	medium	good	fair	30.4-33.2
montana	good	medium	good	fair	25.8
nivea (Calist.)	good	heavy	good	poor	26.1
bakeri	good	heavy	good	poor	25.6
douglasii	good	heavy	good	poor	24.0-29.8
sulphurea	good	heavy	fair	poor	
versicolor	fair	heavy	fair	poor	30.9
parishii	poor	medium	good	good	33.3
floccosa	good	light	light	poor	28.3
rosea	fair	heavy	good	poor	20.0-21.7
pumila	fair	medium	light	good	
striata	fair	heavy	light	poor	29.0
bellingeriana	poor	medium	light	good	

D. Cuphea. Analyses of two samples of Cuphea extend the range of composition of the oils produced by the genus. In oil from C. painteri and C. hookeriana, the major components are C<sub>8</sub>, about 70 percent, and C<sub>10</sub>, about 20 percent, in contrast to the oils of C. llavea, 85 percent C<sub>10</sub>, and C. carthagenensis, 57 percent C<sub>12</sub> and 18 percent C<sub>10</sub>.

	C <sub>8</sub>	C <sub>10</sub>	C <sub>12</sub>	C <sub>14</sub>	C <sub>16</sub>	C <sub>18</sub>	CPR
<u>C. painteri</u>	73	20	0.2	0.3	1.5	4.4	Fair
<u>C. hookeriana</u>	65	24	0.1	0.2	2.1	8.7	Poor

### E. Erucic acid oils.

1. Analyses at NU. Among the 60 Cruciferae samples analyzed by GLC, only 8 species produced oil containing 50 percent or more of acids longer than C<sub>18</sub>.

	<u>&gt; C<sub>18</sub></u> <u>%</u>	<u>C<sub>22</sub></u> <u>%</u>	<u>CPR</u>
<i>Crambe abyssinica</i>	69-50	62-41	Excellent
<i>Sinapis alba</i>	63	52	Good
<i>Brassica tournefortii</i>	61	51	Good
<i>Sinapis arvensis</i>	57	40	Good
<i>Carriichtera annua</i>	56	50	Good
<i>Hirschfeldia incana</i>	51	38	Good
<i>Crambe cordifolia</i>	51	36	Good
<i>Thlaspi arvense</i>	50	36	Excellent

None of the oils are richer in erucic acid than Crambe abyssinica, but three are comparable to the better rapeseed oils.

### 2. Analyses at Montana State College under contract.

a. Brassicas. One hundred ninety-nine samples have been analyzed by gas chromatography, 170 representing the genus, Brassica. Some selections of B. campestris have shown erucic acid content as high as 52 percent. It is believed that selections could be made of plants better adapted to Montana conditions and giving improved yield of seed.

Some vigorous plants of oriental yellow mustard indicate that selections could be made to give higher oil content and higher erucic acid content than the parent stock. However, the highest erucic acid content, 28 percent, leaves much to be desired.

b. Non-Brassicas. Of 24 species other than Brassica, only five produced oils containing over 45 percent of acids longer than C<sub>18</sub> and only 2 contained less than 10 percent longer than C<sub>18</sub>.

	<u>&gt;C<sub>18</sub></u>	<u>C<sub>22</sub></u>	<u>Linolenic</u>	<u>CPR</u>
Iberis amara	71-59	60-54	1-4	Good
Alyssum alyssoides	58	46	8	Good
Lepidium campestre	54	41	3	Good
Sinapis alba	49	36	12	Good
Raphanus sativus	46-32	33-19	6-14	Good
Matthiola bicornis	7	2	53	Good
Berteroa incana	4	trace	49	Excellent

F. Petroselinic acid oilseeds. A need has for some time been evident for an analytical procedure for differentiating and quantitatively determining petroselinic and oleic acids when they co-occur in a seed oil. This topic received some discussion at last year's new crops meeting.

Preliminary studies indicate the possibility of using  $\text{KMnO}_4$ - $\text{KIO}_4$  oxidation of the mixed fatty acids from an oil, followed by gas chromatography for this purpose. The following standardization procedures were used:

(1) Correction factors were tentatively established for varying detector response to the major expected oxidative cleavage products: Pelargonic, lauric, adipic, and azelaic acids. The response (on a weight basis) varied considerably (0.63 to 1.98 in Apiezon and .87 to 1.21 in Resoflex) for the different compounds, and furthermore showed some variation when different percentages were contained in a mixture.

(2) Tentative correction factors were also set up for recovery of oxidation products from known mixtures of oleic and petroselinic acid (using methyl myristate as internal standard and assuming no loss of standard):

	<u>Recovery</u> <u>%</u>		<u>Recovery</u> <u>%</u>
Pelargonic	66	Adipic	82
Lauric	81	Azelaic	94

Since recoveries are better for the dibasic acids, calculations will be based on their values, using the appropriate correction factors developed. Since azelaic acid will also be derived from polyunsaturated  $\text{C}_{18}$  acids, an additional correction will be needed on the basis of the linoleic and linolenic contents of the original oil.



Precautions must be taken to minimize or prevent loss of components during recovery of the oxidation products and in preparing esters for analysis. Optimum procedures have not yet been developed and the analysis has not yet been applied to many Umbelliferae oils. However, the method will probably serve adequately for screening.

G. Sunflower. Sixteen samples of sunflower seed produced in Texas by Dr. M. L. Kinman, CR, were analyzed for oil and protein content and for the fatty acid components. Two replications of two Texas lines and six introductions from Russia were analyzed and the averages for each line are reported below:

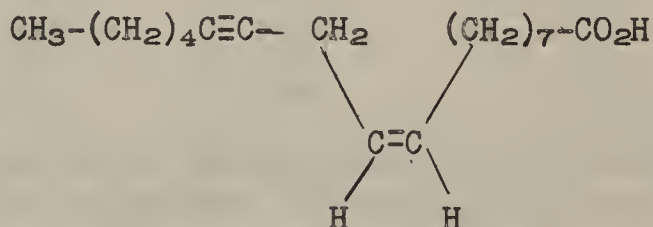
	<u>Oil</u>	<u>Protein</u>	<u>Linoleic</u>	<u>Oleic</u>	<u>Iodine Value</u>
T 55001	32.8	17.4	48.4	42.8	117.7
T 56002	34.4	16.6	36.5	54.3	105.6
Pl 265099	46.2	18.2	36.4	54.0	107.4
Pl 265100	42.0	18.9	31.6	59.1	103.2
Pl 265101	44.6	19.2	37.4	53.5	110.5
Pl 265102	43.8	19.1	44.6	45.4	113.4
Pl 265103	42.7	19.9	42.8	47.4	112.1
Pl 265104	38.4	17.8	52.5	37.8	120.4

Ultraviolet absorption indicated from 0.5 to 1.0 percent of apparent dimorphelic acid in all oils.

## II. Organic Characterization Research

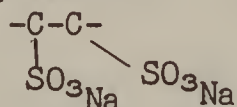
### A. Characterization work completed.

1. Crepis foetida L. ssp. rhoeadifolia (M.B.) Sch et K. (CPR good; family Compositae) seed oil (23.9 percent) contains 60 percent of a new acetylenic acid, cis-9-octadecen-12-ynoic acid. The seeds weigh 0.6 g. per 1000 seeds.



Alkali isomerization (A.O.C.S., official method, 25 minutes, 6.6 percent KOH in ethylene glycol) of Crepis oil showed 88 percent of conjugated triene. An isomerization sample removed after 3 minutes of heating showed 140 percent of conjugated triene. The oil contains essentially no trienoic acid before isomerization. Although monoacetylenic,

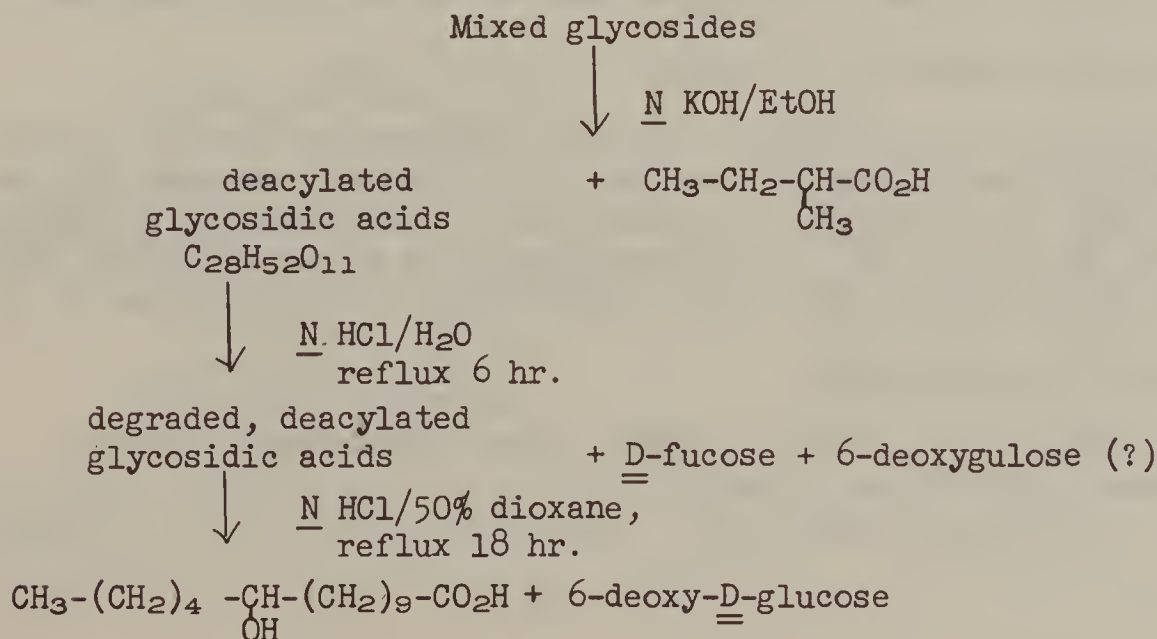
conjugated acetylenic, and conjugated enynic acids have been found in nature, this new acid represents a type which has never before been reported. This dehydrolinoleic acid isomer of linolenic acid is unusual in that the acetylenic bond is isolated from the other unsaturation by a methylene group. The new acid may have value both for basic and applied research. It would be a good substrate for studies of relative reaction rates of acetylenic vs. olefinic unsaturation, active methylene comparison studies, oxidative studies, isomerization studies, comparison with essential fatty acids, etc. Many interesting derivatives should be possible by taking advantage of the varying reaction rates of nucleophilic and electrophilic reagents with olefinic and acetylenic bonds. For example, hydration in presence of mercuric salts may provide a ketone in good yield; two moles of  $\text{NaHSO}_3$  might add to give a compound containing the grouping  $\text{H H}$  , etc. Through arrangements made by SU, the



methyl ester of the acetylenic acid was tested to a very limited extent for antimicrobial activity. Good inhibition was shown against two of the four microorganisms used (active against Trichophyton rubrum and Microsporum nanum). This lead requires following up.

2. The seed oils from Marshallia caespitosa Nutt. (22.8 percent; family Compositae), Alyssum maritimum (L.) Lam. (31.3 percent, family Cruciferae), and Selenia grandis Martin (18.3 percent, family Cruciferae) contain cis-11-eicosenoic acid as the major component, 44 percent, 42 percent, and 58 percent, respectively. Marshallia is the first member of the Compositae family found to yield seed oil with a significant quantity of this acid. The acid could be obtained by fractional distillation. The possible utility of this acid most probably would parallel the uses of oleic and erucic acid, thus providing derivatives intermediate to oleic and erucic acid derivatives.

3. Ipomoea parasitica (HBK) Don. (CPR fair; family Convolvulaceae) seed oil (18.6 percent) contains about 35 percent of a glycosidic material. The following scheme, a modification of the scheme presented last year, was used to fractionate the components.





The seed would be a possible source of hydroxy acids or unusual sugars if developed as a crop or the glycosides themselves may possess utility.

4. Nature of a yellow pigment in the seed of Dalea nutans (CPR good). This legume grows as a low shrub in Mexico. The seed contains a yellow pigment found in capsule-like areas on the seed coat. The color can be easily extracted with methanol. The whole seed contained about 0.28 percent (2800. P.P.M.) of the pigment expressed as carotene. It was thought that the plant might be a source of pigment for animal feeds. Further study of the pigment showed most of it (> 90%) to be a mixture of flavonoids with maximum absorption at lower wavelengths than the maximum for carotenoids and that the maxima varied with the pH of the solution. Methanol extracts from ten other closely related Dalea species were all relatively quite low in pigment content.

#### B. Characterization work in progress

1. Characterization of the unusual diene fatty acid in seed oil of several species of the mint family (Labiatae) is still in progress. The oil (35%) from the accession on which we are working, Leonotis nepetaefolia (CPR fair) contains about 15 percent of this acid. Present studies indicate that it is an optically active acid,  $[\alpha]_D^{28} -47.3$ , containing an allenic grouping and possible branching. Permanganate-periodate oxidative cleavage of the unsaturated acid yields lauric acid and gas chromatographic analysis of the free acids resulting from the cleavage indicates lauric and acetic acids. Weight of seed--2.0g./1000 seeds.

2. Research on Penstemon (fam. Scrophulariaceae) seed lipid unsaponifiabiles has been initiated. The accession selected, P. spectabilis (CPR good), 26.6 percent oil, contains 16 percent of unsaponifiabiles. The unsaponifiabiles fraction has been separated into three major but complex fractions. One of the many components has been obtained chromatographically pure (T.L.C.). Weight of seed--0.8g./1000 seeds.

3. Helichrysum species appear to have some of the same en-ynoic acid as in Crepis foetida. Another Crepis species (Crepis occidentalis, CPR fair) has a very polar unknown acid which appears on the GLC curve. Study has been initiated on some Helichrysums and on the new Crepis species.

#### III. Selected Species That May Merit Further Study:

A. Tetraenes, nonconjugated. Only two samples contained as much as 5 percent tetraene. Both were in the borage family, and the highest concentration of tetraene, 13 percent, was in oil from Echium plantagineum, which had an I.V. of 197. E. plantagineum is a biennial weed of the southern Mediterranean with fair to good CPR. See also section B. 3.

#### B. Trienes, nonconjugated.

1. Five samples were found to contain over 55 percent apparent linolenic acid.



	<u>%</u>	<u>I.V.</u>	<u>CPR</u>
Euphorbia sp.	64	200	
Monarda punctata	63	208	Fair
Verbena macdougalii	60	199	Good
Disanthus cercidifolius	58	182	Poor
Euphorbia bicolor	58	195	Good

2. Two samples from Thalictrum were found rich in the t-5,c-9,c-12-octa-decatrienoic acid.

	<u>%</u>	Seed Composition, % <u>Oil</u>	<u>Protein</u>	Seed Size <u>g./1000</u>	CPR
T. minus	46	41	29	1.7	Fair
T. lucidum	42	38	33	0.4	Fair

3. Three samples from borage species showed the presence of the probable 6,9,12-octadecatatrienoic acid.

	<u>I.V.</u>	<u>6,9,12-triene</u>	<u>9,12,15-triene</u>	<u>Tetraene</u>	<u>CPR</u>
Echium plantagineum	197	10	34	13	Fair to Good
Anchusa capensis	157	10	17	3	Fair to Good
Onosma stellulatum	195	4	41	9	Fair

C. Trienenes, conjugated. Conjugated trienes were found in oils from the Calendulae (section I. A.), and from Mallotus philippinensis. Ultraviolet absorption indicated 55 and 57 percent of conjugated acid (as eleostearic) in two samples of Mallotus (kamala) oil extracted by ethyl ether. GLC analyses have not yet been made, and we have as yet no definite evidence of the 18-hydroxyeleostearic acid reported in the literature. The oil is unusual in that extraction with petroleum ether for 6 hours removes only half as much oil as ethyl ether. The plant is a woody shrub with poor CPR.

D. Dienes. Ten samples were found to produce oil with at least 75 percent of nonconjugated dienoic acids.

	<u>CPR</u>
Hydrangea paniculata	84
Campanula persicifolia	82
Pulsatilla vulgaris	81
Urtica dioica	81
Philadelphus grandiflora	80
Helichrysum fulgidum	79
Vitex agnus-castus	78
Adenophora potaninii	77
Oenothera drummondii	76
Stachys olympica	75

E. Monoenes. (Omitting Cruciferae and Limnanthaceae). Five samples contained oils rich in monoenoic acids. Peucedanum capense and

Ammi visnaga are Umbelliferae and presumably contain both petroselinic and oleic acids. Camellia japonica and Euphorbia lathyris are reported in the literature to contain oleic acid in amounts agreeing with our results. Sapindus mukorossi contains 16 percent of C<sub>20</sub> monoene and 55 percent C<sub>18</sub> monoene. The presence of both these acids is not unexpected in the Sapindaceae.

	<u>%</u>	<u>CPR</u>
Peucedanum capense	86	Poor to fair
Camellia japonica	84	Poor
Euphorbia lathyris	84	Good
Ammi visnaga	80	Fair
Sapindus mukorossi	72	Poor

F. Oils containing at least 10 percent of unsaponifiable matter. An ion-exchange procedure has been adopted to separate unsaponifiable matter from fatty acids. The procedure was standardized during the year and is believed to be giving good results on oils that do not contain essential oils. Reanalysis of three oils reported previously give Daucus pusillus about 30 percent instead of 66 percent, Schinus molle 14 percent instead of 23 percent, and Dactylis glomerata, 9 percent instead of 12 to 18 percent. Other analyses follow:

	<u>Family</u>	<u>%</u>	<u>CPR</u>
Sagittaria sagittifolia	Alismataceae	69	Poor
Tagetes lucida	Compositae	26	Good
Glycyrrhiza glabra (in pod)	Leguminosae	26	Fair
Perezia sp.	Compositae	25	
Penstemon campanulatus	Scrophulariaceae	20	Fair
Iris germanica	Iridaceae	20	Fair
Iris schachtii	Iridaceae	19	Fair
Sambucus canadensis	Caprifoliaceae	19	Poor
Ceratopetalum gummiferum	Cunoniaceae	17	Poor
Vitex agnus-castus	Verbenaceae	17	Poor
Ammoselinum popei	Umbelliferae	16	Good
Magnolia grandiflora (fruit flesh)	Magnoliaceae	12	Poor
Dyssodia (cf. hartwegi)	Compositae	11	Good

G. Acetylenic oils. Oil from Crepis foetida (Compositae) (CPR Good) was found by GLC to contain 60 percent of an unidentified acid, since identified as cis-9-octadeca-12-ynoic acid. This acid occurs (GLC evidence) in Helichrysum bracteatum (15 to 20 percent) (CPR Good), and in Matricaria capensis (6 percent) (CPR Fair). The identity in these other sources should be proved more rigorously. A second species of Crepis, C. occidentalis (CPR Fair), contains 9 percent of this acetylenic acid and 12 percent of a more polar unknown.

H. Major unknown components by GLC. Flacourtia indica (CPR very poor), 21 percent probably chaulmoogric acid. Aster tanacetifolius (CPR Good), 12 percent probably unusual C<sub>16</sub> monoene. Heterotheca latifolia (CPR Good),



10 percent probably unusual C<sub>16</sub> monoene. Athanasia tomentosa (CPR Fair), 26 percent unknown, 6 percent oil content. Ammoselinum sp. (CPR Fair), 11 percent.

I. High-protein seeds. Six samples were found to contain at least 50 percent crude protein. Five of these, with crude protein ranging from 50 percent to 71 percent, are members of the Proteaceae and probably give a falsely high figure because of a high arginine content. The other sample is Onobrychis aurantiaca (CPR Fair), a legume. This seed contains 57 percent crude protein and 13 percent oil. The protein contains 4.9 percent lysine and 1.7 percent methionine and no canavanine. The seed size is 5.7 grams per 1000 seeds. Complete amino acid analysis is provided later in the report. Two related species from Turkey were also found to have high protein seeds, as follows:

	Protein %	Oil %	CPR
<u>O. argyrea</u>	49	17	Fair
<u>O. hypogyrea</u>	48	14	Fair

In addition to the above, a total of 25 species has been found since beginning of the program whose seed (obtained without excessive removal of extraneous accompanying tissues) contained 45 percent or more protein. These might be further investigated should their composition be judged to warrant it.

J. Samples unusually rich in total oil plus protein should be especially attractive for production and processing if agronomic and utilization factors are promising. Listed below are those samples encountered since the beginning of the program in which the component analyzed contained at least 75 percent of oil plus protein. Trees, shrubs, vines, and assigned oils (Calenduleae, castorbeans, and oils with more than 25 percent erucic acid) have been omitted. Some indication of oil composition is given where possible. Hard or fibrous outer tissues, sometimes in very large amounts, were removed to provide a "seed" component suitable for analysis. Simple illustrations of this preparative step might include operations such as shelling peanuts and walnuts, threshing wheat, removing pod and sometimes seed coat from soybeans. In practice, such simple operations were extended to many unique materials by the best methods we could devise.



## Component Analysis

Name	Family	CPR	Component	Wt./ 1000	(Nx6.25) % protein	% Oil	Sum	Oil Data
Onosmodium occidentale	Boraginaceae	Fair	S-SC	18	36	56	92	
Jatropha macrorhiza	Euphorbiaceae	Fair	S	101	36	53	89	
Teucrium creticum	Labiatae	Fair	S	0.9	37	52	89	
Martynia sp.	Martyniaceae		S-SC	54	32	56	88	
Onosmodium molle	Boraginaceae	Fair	S-Sc	13	33	54	87	I.V. 185, 18:4; 18:3
Scaevola plummieri	Goodeniaceae	Poor	S-SC	61	19	67	86	50% 18:2
Chrozophora tinctoria	Euphorbiaceae	Good	S	3	37	49	86	
Echinaceae angustifolia	Compositae	Fair	S+P	2	49	37	86	65% 18:2
Cnidioscolus angustidens	Euphorbiaceae	Poor	S	51	42	44	86	
Martynia louisianica	Martyniaceae	Good	S-SC	20	25	60	85	
Xanthium strumarium	Compositae	Fair	S	79	49	36	85	65% 18:2 (lit.)
Arctotis grandis	Compositae	Good	S-SC	0.8	36	48	84	
Gundelia tournefortii	Compositae	Good	S	66	44	39	84	
Jatropha spathulata	Euphorbiaceae	Fair	S-SC	127	26	58	84	50% 18:2
Isomeris arborea	Capparidaceae	Fair	S-SC	44-41	41-37	45-42	83-82	
Mundtia spinosa	Polygalaceae	Poor	S	15-12	31-30	52-44	82-75	50% 18:1
Actinomeris alternifolia	Compositae	Fair	S	5	43	39	82	
Gaura neomexicana	Onagraceae	Good	S	1	31	48	80	
Asclepias engelmanniana	Asclepiadaceae	Fair	S	6	42	38	80	
Cakile edentula	Cruciferae	Good	S	10	31	49	80	17% 22:1
Xanthium pensylvanicum	Compositae	Fair	S	60	41	38	79	
Xanthium sp.	Compositae		S	44	44	34	78	
Martynia parviflora	Martyniaceae	Good	S-SC	9	30	48	78	
Cardopatum corymbosum	Compositae	Fair	S+P	10	43	34	78	62% 18:2
Neslia paniculata	Cruciferae	Good	S	1	31	47	78	23% 20:1; 7% 22:1
Boreava orientalis	Cruciferae	Good	S	8	36	41	77	11% 20:1; 14% 22:1
Liatris punctata	Compositae	Fair	S	32	47	29	77	60% 18:2
Cakile maritima	Cruciferae	Fair	S	4	33	44	77	25% 22:1
Nemesia suttonii	Scrophulariaceae	Good	S	0.2	29	48	76	62% 18:2
Hymenoclea monogyra	Compositae	Poor	S	0.3	39	37	76	74% 18:2
Flourensia pringlei	Compositae	Poor	S+P	26	46	31	76	60% 18:2
Schimpera arabica	Cruciferae	Good	S	1	37	39	75	11% 20:1; 11% 22:..

S = Seed

S+P = Seed plus pericarp

S-SC = Seed minus seed coat

K. Selected palmitic acid sources. In January of 1959 SU's Mr. Hopper solicited industrial opinions regarding fatty acids that might be advantageously sought in new oilseeds. Mr. J. L. Troutt, chief chemist of Emery Industries, wrote him, "Since so much cottonseed soapstock now finds its way into animal feeds, there is a growing need for new cheap sources of palmitic acid." We list our richest herbaceous sources of palmitic acid below.

	<u>Palmitic</u>	<u>Stearic</u>	<u>Oleic</u>	<u>Linoleic</u>	<u>CPR</u>
<i>Tithonia speciosa</i>	37	25	7	14	Good
<i>Cosmos</i> sp.	32	5	11	50	
<i>Lavatera trimestris</i> <sup>2/</sup>	31	6	30	15	Good
<i>Bidens</i> sp.	30	4	10	55	
<i>Alternanthera sessilis</i>	29	3	25	40	Poor
<i>Medicago tribuloides</i> <sup>1/</sup>	28	3	14	24	Fair
<i>Malachra capitata</i>	27	3	7	59	Fair
<i>Lavatera</i> sp.	27	3	14	53	
<i>Dahlia variabilis</i>	26	2	18	49	Fair

<sup>1/</sup> 26 percent linolenic

<sup>2/</sup> Two other *L. trimestris*, 18 to 19 percent palmitic.

Cottonseed oil contains about 20 to 25 percent palmitic.



## PRELIMINARY DEVELOPMENT WORK ON OILSEEDS

## I. NU - Erucic Acid Oilseeds

A. General. From the 1962 crop a total of between 2 and 2-1/2 tons of crambe seed was received from Montana, Nebraska, and Texas through CR.

B. Variation in gross composition with location and year. Data are as shown in the following table.

Analytical Data on Crambe Seed

Location	Crop Year	Percent Constituent in			
		Seed and Pericarp		Dehulled Seed	
		Oil	: Protein	Oil	: Protein
Alaska	1961			48.3	27.4
Beltsville, Maryland	1957			35.5	31.5
Beltsville, Maryland	1961	31.4	26.4		
Indiana	1959	30.4	27.2	39.7	33.4
Iowa	1959	32.5	24.4		
Manitoba, Canada	1961	36.8	23.0	46.3	27.9
Minnesota - early	1959	35.0	21.9		
"	1959	35.9	21.9		
Minnesota - medium	1959	34.9	23.1		
Minnesota - late	1959	31.6	23.8		
Minnesota	1960	30.8	25.8	39	31.5
Minnesota	1960			40.0	32.8
Minnesota	1961	32.6	23.6	42.2	30.4
Missouri	1959	27.7	28.8		
Montana	1960	26.6	19.8	45.0	27.6
"	1960	24.8	20.5	42.8	29.6
"	1960	28.2	20.2	46.4	27.5
"	1960	29.6	21.3	45.4	27.8
Nebraska	1957			48.1	25.0
"	1962			47.3	24.9
"	1962	31.7	24.6	40-42	29-32
North Carolina	1961			40.5	29.9
South Dakota	1961	33.1	24.9	42.2	28.8
Texas	1960	33.8	25.0		
Texas	1961	39.7	24.7	45.7	26.8
Texas	1962	31.3	23.9	41.4	29.9
Wyoming	1961			48.3	25.6

Crambe oil varied in erucic acid content from 61 to 54 percent, excepting for the one sample from Alaska which contained only 40 percent.

### C. Studies of crambe seed lipase and myrosinase enzymes.

#### Crambe lipase activity:

The stability of crambe oil to lipolysis in flaked seed was studied as a function of moisture content of the stored flakes. In contrast to Vernonia anthelmintica lipase, crambe lipase is inactive under dry storage (5 to 7 percent moisture) at room temperature.

Method--The pericarp was removed, and the seed flaked between smooth rolls. The flakes were spread out in thin layers in desiccator jars, and stored in a nitrogen atmosphere to inhibit microbial decomposition. Moisture content was increased either by saturating the atmosphere with water or by vacuum infiltration, after which the flakes were again placed in a nitrogen atmosphere. At intervals samples of the flakes were analyzed for moisture content, and the oil extracted for titration of free fatty acids. At the higher moisture contents, hexane does not completely extract the oil. Diethyl ether was used to extract the residual lipid (figure 1). Results reported below represent the total oil--hexane extract plus the diethyl ether extract.

Results--Figure 2 shows the stability of the crambe oil to lipolysis when the flakes are stored in a dry atmosphere. At 12 to 15 percent moisture, lipase appears to be inactive (figure 3). This experiment had to be terminated on day 20 because of an overgrowth of mold.

If the flakes are stored in a water-saturated atmosphere, lipase activity develops slowly, and hydrolyzes the seed oil in about 7 weeks (figure 4). Raising the moisture content above 20 percent more promptly by vacuum infiltration shortens the ~~time~~ necessary for the appearance of lipase activity (figure 5). Thus, it appears that a critical moisture content is necessary to initiate lipolysis. The oil is stable at 12 to 13 percent moisture in the flakes, but is rapidly hydrolyzed at moisture contents greater than 20 percent.

Conclusions--The lipase activity of crambe seed should not have unfavorable effects on its storage and processing. The critical moisture contents for lipase activity and molding of seed have not been precisely established, but appear to be between 13 and 20 percent.

#### Crambe myrosinase activity:

Crambe abyssinica seed contains the enzyme system myrosinase, which hydrolyzes thioglucosides such as sinigrin and sinalbin. On hydrolysis, thioglucosides of this class give rise to the mustard oils or related products, glucose, and inorganic sulfate. The enzymatic reactions are complex and have not yet been completely elucidated. On crambe seed enzyme in particular, nothing at all appears in the literature.

Myrosinase has been separated from crambe seed as a water-insoluble particulate fraction under conditions which lead to a water-soluble myrosinase from white mustard seed (Sinapis albus). This may be





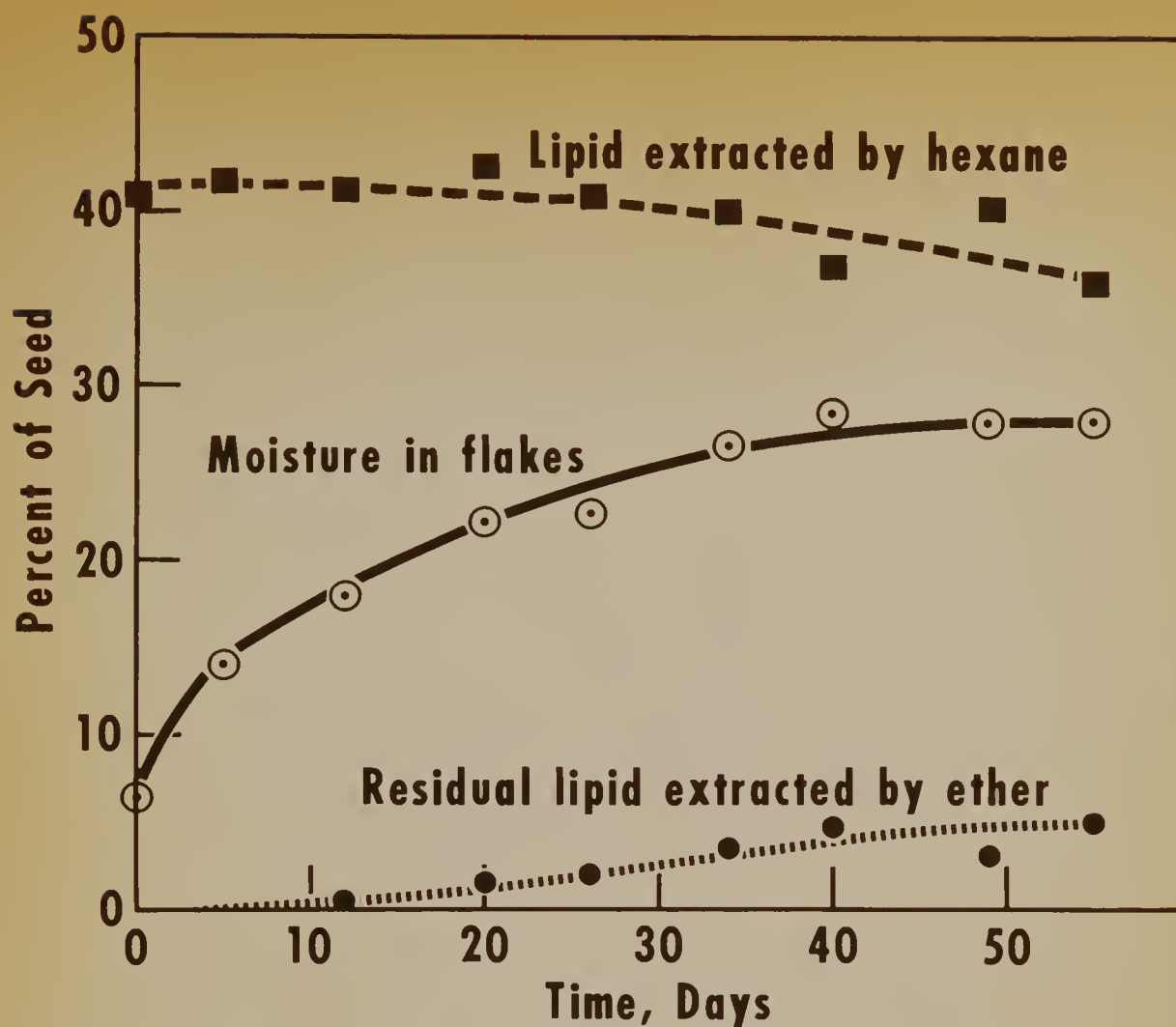


Fig. 1. Relation of extractability of lipid material to moisture content of flaked Crambe seed

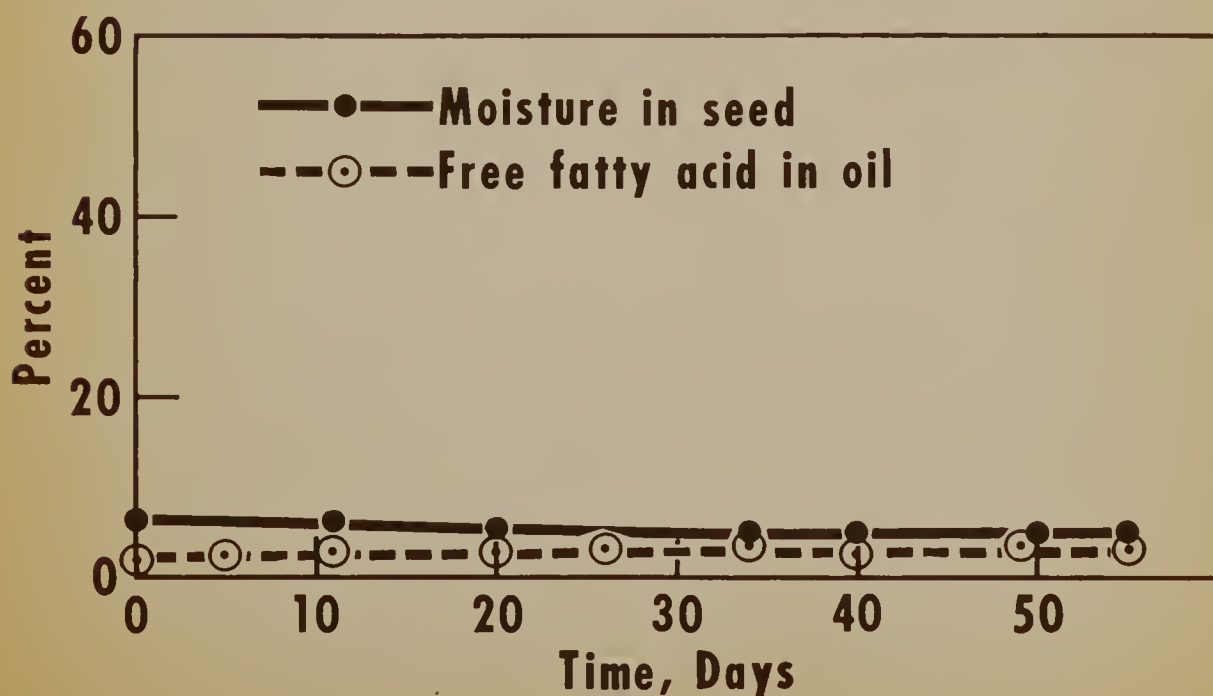


Fig. 2. Lipase activity in flaked Crambe seed: dry atmosphere





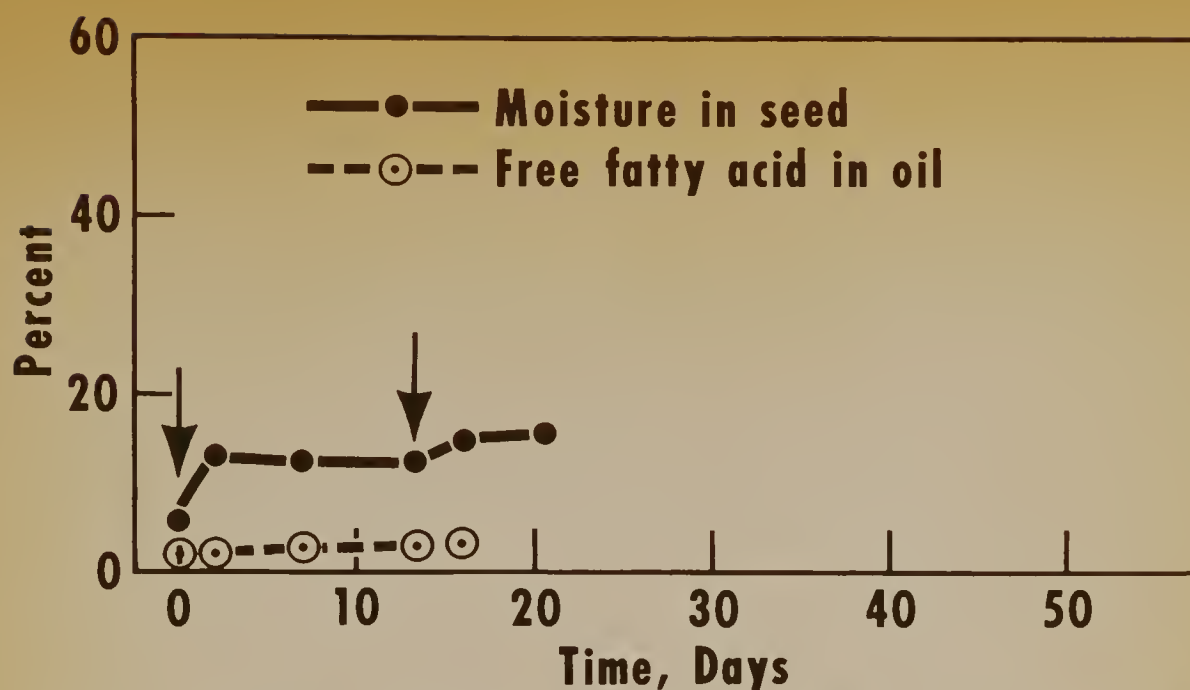


Fig. 3. Lipase activity in flaked Crambe seed: 12-15% moisture content. Water added by vacuum infiltration at times indicated by arrows

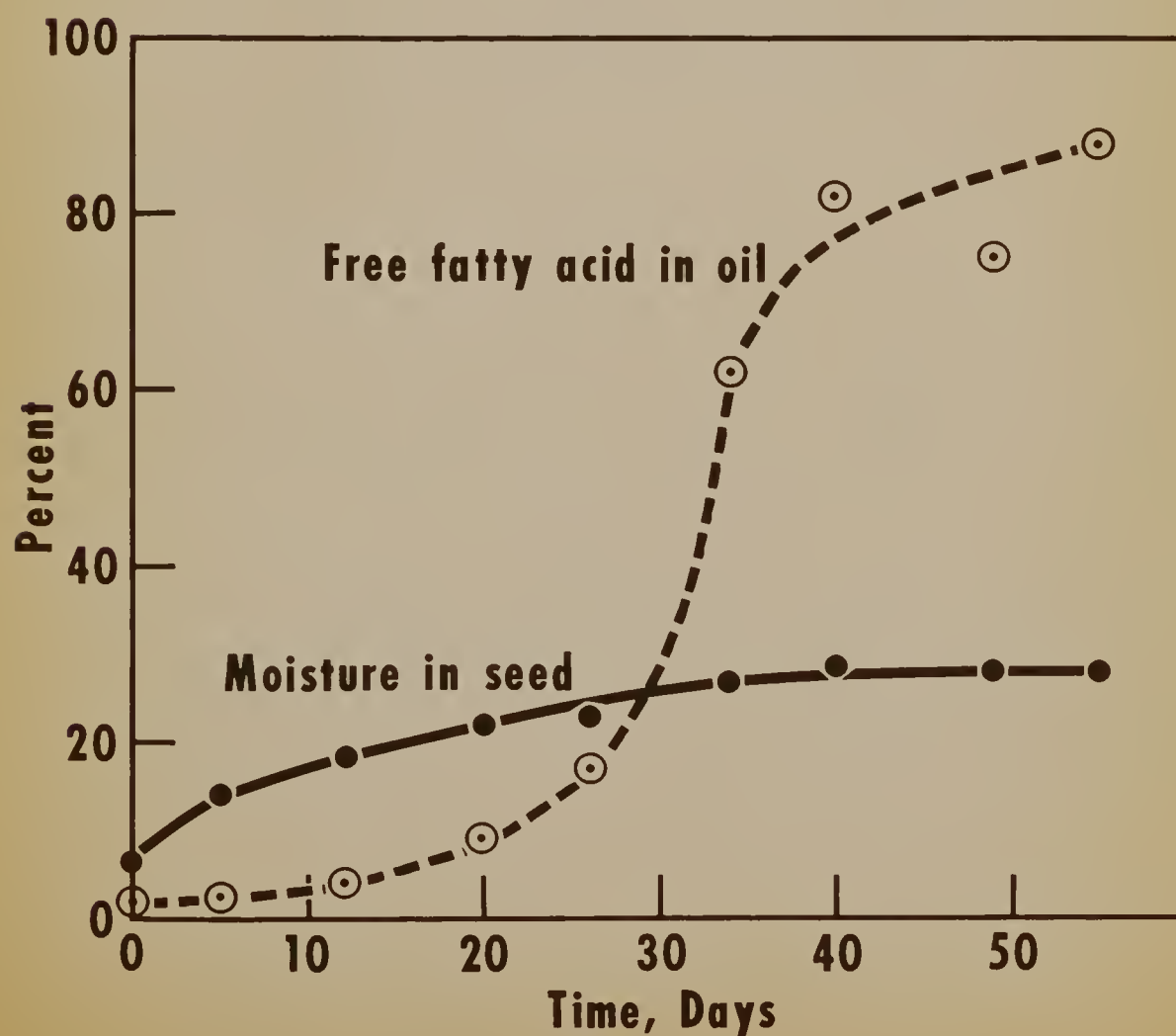
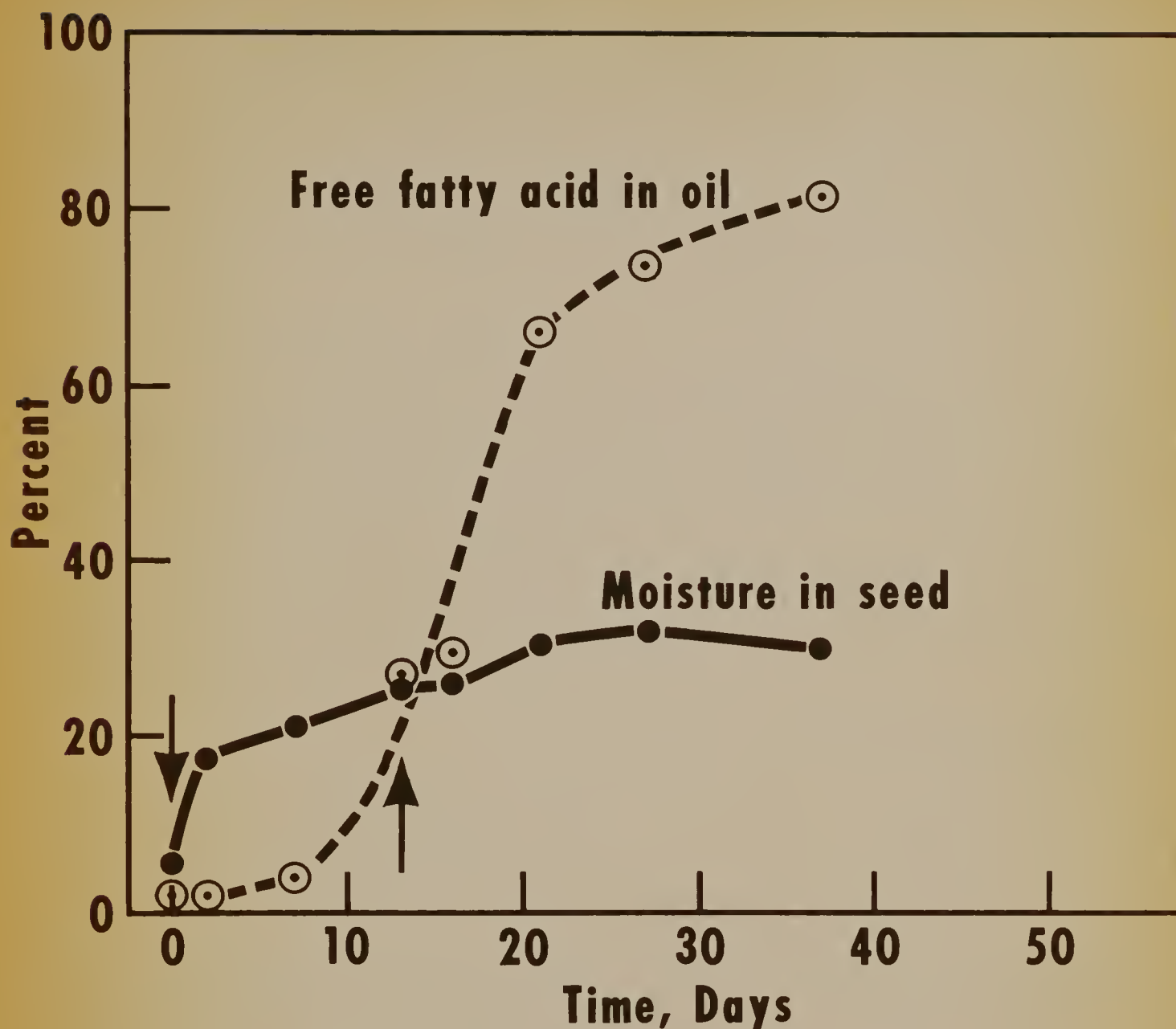


Fig. 4. Lipase activity in flaked Crambe seed: water-saturated atmosphere







**Fig. 5. Lipase activity in flaked Crambe seed: high moisture content. Water added by vacuum infiltration at times indicated by arrows**





indicative of a fundamental difference between the mustard and crambe thioglucoside-splitting enzymes. The crambe myrosinase is activated approximately twofold by 0.001 M ascorbic acid.

Processing of crambe seed to form a wholesome meal for feeding involves the thioglucoside(s) of the seed and their hydrolytic products. A knowledge of the effects of heating, of changing pH, or of oxidizing conditions on the myrosinase present will be helpful in understanding and guiding the processing of crambe seed. By analogy to other crucifers, a change in pH or temperature during processing may lead to the formation of products different from the expected isothiocyanates or oxazolidinethiones. In certain cases normal thiocyanates and inorganic thiocyanate ion have been detected.

#### D. Characterization of crambe oil.

Oxidative stability--The Olcott-Einset method of determining oxidative stability was applied to crude and refined crambe oil, crude and refined soybean oil, and water-washed linseed oil. One-gram samples of oil in 30 ml. beakers were heated at the selected temperature (80° C.) and weighed at intervals. An increase of weight of 0.4 percent was taken as the end of the induction period. Results were generally good, but those for refined crambe oil were quite variable. The varied history of the oils prevents precise comparison of the results, but it seems definite that crambe crude and refined oils are more stable than soybean oil, and both are more stable than linseed oil.

	<u>Induction period, hrs.</u>
Crambe crude (4)	183-200
Crambe refined (10)	53-115
Soybean crude (4)	100-134
Soybean refined (4)	37-43
Linseed water-washed (4)	10-12

Viscosity--The viscosity of crambe oil was determined with the Gardner Bubble Viscometer at room temperature, 26.5° C. Linseed, soybean and Lunaria oils were measured for comparison. The results are in fair agreement with results obtained by Dr. Miwa with the Cannon-Fenske Viscometer at 25° C.

	<u>Gardner</u>	<u>Cannon-Fenske</u> <u>Poise</u>
Linseed	A to A-1 (0.41 poise)	
Soy	A (0.50 poise)	
Crambe, crude	C (0.85 poise)	
Crambe, refined	C (0.85 poise)	.750
Lunaria	D (1.00 poise)	.901

Glyceride structure--(1) Separation of glycerides by cooling of the oil has been investigated on a laboratory scale. Cooling of crambe oil to 8 to 11.5° C. for 12 days does not cause solidification of the oil. However, cooling to 4.5 to 6° C. for 7 days causes the solidification of



40 percent of the oil. There is no essential difference between the fatty acid composition of the solids (GLC analysis) and that of the liquid oil. Significance: Transportation of crambe oil in winter may subject the oil to low temperatures; solids which may separate are of the same composition as the bulk liquid.

(2) Experiments are in progress to separate the glycerides of crambe oil by countercurrent distribution between pentane-hexane and nitroethane-furfural. Because of the high expected content of dierucyl glycerides, the differences between glyceride components is minimal. Good separation has not yet been accomplished. Significance: Knowledge of the component glycerides is a necessary first step in devising methods of concentrating selected fatty acids.

#### E. Laboratory Studies on Crambe Seed Meal.

Preparation of hexane extracted meal and fractionation of the meal for feeding and isolation work. For this work, a 7-pound laboratory preparation of the meal was made by room temperature hexane extraction of the oil by percolation of the hexane through the flaked crambe meal in large cone-shaped extractors. The meal, after eight extractions, drying, grinding, and reextracting, had a residual oil content of 0.2 percent. For analytical data on the meal, see Table I, page 26.

Of the solvents tested to remove the thioglucosides from the meal acetone-water 80-20 (V/V) was one of the most effective. Such a room temperature extraction was made on samples of the above meal for isolation work and for feeding trials. Three fractions were obtained: the aqueous acetone extracted meal, a water insoluble fraction obtained as the acetone was removed from the aqueous acetone extract, and an aqueous acetone and water soluble fraction. The aqueous acetone-extracted residue (75% of meal) was high in protein. The water soluble extract (20% of meal) contained nearly all the recovered thioglucosides. The water insoluble acetone extract 3 percent of meal contained with other substances an unusual protein and some phosphatide-like material. For analytical data on these fractions, see last three columns of Table I, page 26.

Aqueous acetone extracted residue meal fraction. In addition to higher crude protein content, this fraction was of better nutritional quality based on amino acid composition (Table I). Lysine content was only slightly lower than soybean meal and methionine content was higher. On the basis of short term feeding experiments at WU with rats and mice, this fraction gave close to normal growth response when tested for toxicity on an addis diet. This growth response was obtained without any heat treatment of the meal, a common requirement for optimum growth with soybean and cottonseed meal and an indicated requirement for whole crambe meal as shown by preliminary work by ED at NU with rats fed at WU and at Texas A. and M. with poultry. Further work is indicated to see if this fraction obtained by aqueous acetone extraction of a cold hexane extracted meal can be made a source of high quality high protein concentrate for feeding or food purposes.

Aqueous-acetone-soluble, water-insoluble fraction. This fraction was the source of an unusual crystalline, low molecular weight, prolamine type protein of high purity with respect to nonprotein constituents (see Table I). Of interest is the high cystine, threonine, tyrosine, isoleucine and proline content and the absence of lysine, histidine, and tryptophan. Possible biological activity or relationship of this high sulfur containing protein to thioglucoside formation in the plant, if any, awaits further investigation. Results of initial feeding trials with mice at WU under the conditions of tests which were complicated by the refusal of the animals to eat indicate that the fraction from which this protein was obtained may be more toxic than the aqueous acetone water soluble fraction containing the thioglucosides. This fraction also contained dark brown viscous material, soluble in petroleum ether. Phosphorus and nitrogen content indicated the material to be phospholipid in nature. However, it was soluble in aqueous acetone.

Aqueous-acetone-soluble, water-soluble fraction. This fraction contained very little if any protein. Nearly all the recovered thioglucosides were in the fraction. However, even with the mild conditions of fractionation employed in this experiment, about one-half the thioglucosides could not be found in the fractions obtained. When this fraction was fed to rats and mice at the 1 percent level to an addis diet at WU the animals' growth was significantly below the positive controls.

Thioglucosides and their enzymatic products. Analytical information obtained by different methods all indicated that at least 3 to 4 percent of the hexane extracted meal is thioglucosides. Group separations by ion exchange and qualitative chromatography showed about half the sulfur and 4 percent of the nitrogen of the meal was probably present as thioglucosides.

From the aqueous-acetone, water soluble fraction a crystalline acetyl derivative of the thioglucoside(s) in good yield was obtained. Characterization data from the crystalline acetyl derivative indicated the bulk of the thioglucosides to be similar to or identical with the one which yields vinyl thiooxazolidone (goitrin). Examination of the products obtained from deacetylation and enzymatic hydrolysis of the crystalline acetyl derivative indicated the derivative to be from a mixture of thioglucosides. Attempts to date to further positively identify the major thioglucosides through their enzymatic hydrolysis products have not been successful.

The thioglucosides present in smaller amounts which yield volatile isothiocyanates are at least four in number. Based on paper chromatography of their thiourea derivatives, the volatile isothiocyanates consist of allyl-, 3 butenyl-, 4 pentenyl- or benzyl- and  $\beta$ -phenyl- ethylisothiocyanate. In addition to the above end products, a small but definite amount of thiocyanate ion was obtained from enzymatic hydrolysis. For following the thioglucosides and their hydrolytic products, a thin layer method of chromatography was developed.



Low molecular weight substances in the water soluble acetone extract other than thioglucosides. Amino acid composition of this extract was determined before and after acid hydrolysis. Before acid hydrolysis only 4 percent of the nitrogen in the extract was present as known amino acids and 0.5 percent as ammonia. After acid hydrolysis, only 11 percent of the nitrogen was present as known amino acids but 26 percent was present as ammonia. Obviously, a large amount of nitrogen-containing compounds were present that were decomposed during acid hydrolysis. Before acid hydrolysis, 15 minor unidentified ninhydrin color-producing substances were detected on the elution chart. After acid hydrolysis, most of these peaks were not present. Results similar to the above were obtained with a trichloroacetic acid extract of the meal.

The water soluble acetone extract contained 9 percent of the total nitrogen and the trichloroacetic acid extract contained 10.3 percent. For comparison a trichloroacetic acid extract of soybeans contains 3.2 percent of the total nitrogen and of corn 5.2 percent. An 80 percent alcohol extract of whole corn contains 2 to 3 percent of the total nitrogen half of which is present as known amino acids without acid hydrolysis.

Based on group separations by ion exchange and thin layer and paper chromatography, the crambe meal contains several percent of sugars which were extracted by the aqueous acetone and were present partly as thioglucosides and partly as sucrose which was crystallized from the extract and positively identified.

Based on elution position with the amino acid analyzer and Rf values on chromatography, the meal contains 0.2 g. of ethanolamine per 16 g. of nitrogen.

The aqueous acetone and water soluble extract contained large amounts of a cationic, ultraviolet, fluorescing compound which turned visible yellow in strong alkali. The compound had the same Rf on paper as sinapin, a bitter substance obtained from white mustard. Attempts at crystallization by methods described for isolation of the compound from mustard meal were unsuccessful.

Significance of the above work. The chemical diversity of the lower molecular weight compounds indicated to be present in large amounts in comparison with other seed extracts emphasizes the need for more characterization work to be done on the crambe seed meal. More fundamental knowledge concerning the nature of the compounds present could lead to practical uses. The diverse nature of these substances and the poorly defined nature of the toxic material present indicates that a thorough job of animal testing must be done on the seed meal treated for detoxification in an empirical way, before it is released for use as part of a feeding supplement.

Solubility of crambe meal nitrogen as a function of pH. The hexane-extracted seed meal showed a minimum solubility at pH 3.5-4.5 and pH 6.8-6.0 of 40 and 44 percent, respectively, of the total nitrogen in the meal. Above pH 10.5 from 95 to 100 percent of the nitrogen was solubilized, at pH 1.0-2.0 about 70 percent was in solution.

The solubility of the nitrogen in relation to the pH of the aqueous extract differed from that of soybean, safflower and bean meal in that a larger amount of nitrogen was in solution in the crambe meal at the pH of minimum solubility. Also, the crambe meal nitrogen showed a minimum solubility at two different pH's, one of which was pH 7.5 at which large amounts of soybean meal nitrogen is in solution. Of the large amount of nitrogen in solution in crambe meal at minimum solubility, only about 10 percent (4% of original meal nitrogen) was considered as from the thioglucosides present.

Precipitation of protein at pH 4.2 after solubilization of 96 percent of the nitrogen in the meal at pH 11.8 gave a precipitate containing 36 percent of the nitrogen in the original meal. The remainder of the nitrogen was still in solution.

The data above shows that in the case of crambe meal protein, isolates are not likely to be obtained in a practical way by solubilization at a high pH and precipitation at a low pH as is done with soybeans.



TABLE I

Composition of Crambe Meal Fractions<sup>a/</sup>

	Dehulled hexane extracted crambe meal <sup>b/</sup>	Dehulled soybean meal (for com- parison)	Aqueous acetone extracted crambe meal	Aq. acetone soluble water insoluble fraction	Aq. acetone soluble water soluble fraction
Crude protein %	48.5	55.0	56.2	43.1(98.7) <sup>c/</sup>	21.0
Wt. % of whole meal	100.	...	75.	3. (ca.1.0)	20.
Nitrogen % of whole meal	100.		86.	3.	9.
Volatile isothio- cyanate mg./g.	3.5	...	0.4	1.8 -	3.2
Oxazolidinethione mg./g.	10.3	...	0.6	0.2 -	21.9
Sulfur %	2.2	...	1.2	1.8 -	5.2
Phosphorus %	1.0	...	0.3	1.1(0.0)	0.1
% N as amino acids	76.1		84.8	68.3(92.8)	11.1
% N as ammonia	13.7		10.7	9.1(7.5)	27.2
Amino acids g. per 16 g.N.					
Lysine	6.0	6.8	6.4	0.2(0.0)	0.0
Methionine	1.8	1.6	2.1	0.5(0.4)	0.1
Cystine	2.0	1.6	...	... (8.7) <sup>d/</sup>	...
Isoleucine	2.6	5.1	4.4	7.8(10.7)	0.5
Leucine	6.5	7.7	7.3	3.1(4.2)	0.3
Phenylalanine	4.1	5.0	4.5	2.6(4.0)	0.3
Tyrosine	3.1	3.9	3.2	5.6(8.3)	0.5
Tryptophan		1.3		(0.0)	
Threonine	4.4	4.3	4.6	9.8(13.9)	0.9
Valine	5.2	5.4	6.0	5.8(8.5)	0.6
Histidine	2.6	2.6	2.7	0.0(0.0)	0.1
Arginine	6.8	8.4	7.2	5.4(6.7)	0.5
Glycine	5.4	4.5	5.8	5.5(7.5)	1.6
Alanine	4.2	4.5	4.7	7.0(9.7)	0.8
Aspartic acid	6.1	12.0	6.4	8.2(11.4)	2.8
Glutamic acid	17.0	21.0	18.8	2.9(3.2)	4.1
Hydroxy proline	1.0	0.0	0.7	0.0(0.0)	0.0
Proline	6.4	6.3	7.1	8.2(11.2)	0.7
Serine	3.7	5.6	4.0	3.5(4.6)	0.3

<sup>a/</sup> Air dry basis.<sup>b/</sup> This accession of crambe ran slightly higher in most amino acids in comparison with the first accession analyzed (see last year's annual report).<sup>c/</sup> Data in parenthesis on a highly purified crystalline protein obtained from the fraction.<sup>d/</sup> Minimum value.

## F. Chemical Investigations on Erucic Acid Oils.

Preparation of brassylic acid. Pilot plant: A supply of brassylic acid for preparation of various derivatives has been obtained in four pilot plant runs. The starting material for each of these batches was 10 lbs. of 87 percent commercial erucic acid. The acid was treated with a mixture of ozone and oxygen at about 20° C. using acetic acid as solvent; the ozonides were oxidatively cleaved by heating with hydrogen peroxide. After removal of solvent by distillation, the mixture was further purified in the laboratory by removing most of the pelargonic acid by vacuum distillation. The crude brassylic acid was highly colored and was also vacuum distilled to remove color. GLC analysis of the best run indicated a yield of 75 percent of theory. Further purification by crystallization from 1:1 ethyl acetate:hexane resulted in 55 percent recovery of 94 percent brassylic acid containing only 0.2 percent pelargonic acid by GLC. This material was still slightly colored.

Laboratory ozonolysis of purified erucic acid (96%) in pelargonic acid solvent followed by oxidative decomposition of the ozonides using heat and ozone (2%)-oxygen (not possible in present pilot-plant equipment) gives yields of better than 90 percent of theory by GLC analysis of the reaction mixture. The most difficult problem in recovery of pure brassylic is complete removal of the by-product pelargonic acid without loss of brassylic acid. Several methods of separation have been tried. Both brassylic and pelargonic acids are soluble in aqueous alkali; addition of a soluble aluminum salt precipitates the aluminum salt of pelargonic. Some separation can be achieved by this method but it is not efficient because of occlusion of brassylic acid in the precipitate. Removal of pelargonic by extraction with hexane from an aqueous acetonitrile solution also effects some separation but it is not clean-cut. The most efficient separation found thus far is removal of pelargonic acid by vacuum distillation using a fractionating column although it is difficult to remove the last traces of pelargonic by this means. The best laboratory separation was accomplished using vacuum distillation followed by crystallization from ethyl acetate and resulted in 70 percent recovery of 98 percent brassylic containing less than 0.1 percent pelargonic by GLC.

Erucic acid can be cleaved to form brassylic and pelargonic acids using ammonium periodate and potassium permanganate. This procedure has been successful only in dilute solution containing one gram of erucic in 300 ml. The product acids can be separated from the reaction media without removing or destroying the oxidant. This method of cleavage does not hold much promise unless the reaction can be accomplished in much more concentrated solution.

Brassylic acid esters. The following series of di-esters of brassylic acid were prepared at NU and have now been evaluated as vinyl plasticizers at EU; methyl, ethyl, n-butyl, isobutyl, n-octyl, 2-ethylhexyl and n-decyl. Esters that are liquid at room temperature, ethyl, n- and iso-butyl and 2-ethylhexyl, show excellent compatibility with PVC with the exception of 2-ethylhexyl. This ester developed a light greasy exudate after milling that is not understood. Another sample has been prepared for reevaluation.



The compatible esters show excellent low temperature flexibility superior to a commercial phthalate ester (di-2-ethylhexyl phthalate) and comparable to commercial low temperature plasticizers such as di-2-ethylhexyl adipate, di-octyl sebacate and di-2-ethylhexyl azelate. Di-isobutyl brassylate imparted a slightly higher tensile strength to PVC than any of the above commercial plasticizers. Migration and volatility are also important in low-temperature plasticizers and in this aspect the n-butyl is close to the commercial plasticizers but not quite as good. Light stability of the n-butyl was similar to the commercial adipate and better than the sebacate and azelate. The heat stability of the n-butyl was about the same as the adipate; both failing after 5 hrs. exposure to heat. The azelate and sebacate failed after 6 hrs.

Evaluation on this first series of esters as vinyl plasticizers indicates good potential for the brassylic esters. Other esters are now in process of preparation, they include: n-hexyl, a mixed ester of 2-ethylhexyl and n-butyl, n- and isopropyl, 4-methyl-2-pentyl, 2-methoxyethyl, 2-(2-n-butoxy-ethoxy) ethyl, 2-methylpentyl, 2-n-butoxyethyl and 2-ethylbutyl.

Contact has been made with Wright-Patterson Air Force Base for possible evaluation of some di-esters of brassylic as Gas Turbine engine oils. Some physical measurements on these materials will be made at NU to determine which esters merit evaluation for this use. Sample quantities up to one gallon will be required for evaluation. A British patent (No. 896,436, May 16, 1962) just obtained claims the use of brassylic diesters of aliphatic alcohols with 5, 6 or 7 carbon atoms as major desirable components of lubricant compositions.

Use of brassylic acid in WU IFP process for treating woolens. One pound of brassylic acid was sent to WU for conversion to the acyl chloride for testing in the WURLAN treatment of wool (washable woolens). Brassyloyl chloride was found to be approximately equal to sebacoyl chloride in this process. Sebacoyl chloride is presently the best commercially available acyl chloride for this purpose. If the price of brassyloyl chloride were equal to, or less than that of sebacoyl chloride (\$2-4/lb.), this material would certainly merit further development work. Anticipated usage might be 700,000 lbs., or more, per year. WU has not carried out extensive studies with brassylic acid because of their desire (understandable) to promote the WURLAN process using presently available raw materials.

Oxyethylation of brassylic acid. Objective and significance: Polyglycols and polyoxyethylene derivatives of acids have been sold commercially as surfactants, foam components, and as resins of widely differing molecular weight for diverse industrial uses. It is not known what the effect of an aliphatic chain of 11 methylene groups in the center of such a molecule might be. Hence, the reactions of brassylic acid with ethylene oxide to prepare a series of products having varying amounts of combined ethylene oxide are being investigated.

Accomplishments: In a dimethyl formamide solution, a mole of brassylic acid reacted with approximately 1.5 moles of ethylene oxide to form primarily the diester of ethylene glycol. In the solid state, using alkaline catalysts, the uptake of ethylene oxide was negligible.

However, when a  $\text{BF}_3$ : etherate slurry of brassylic acid was exposed to ethylene oxide, uptake was very rapid after a short incubating period. The propagation of the oxyethylene chain may be quite extensive, since no limit to ethylene oxide uptake was reached in a preliminary experiment. Properties of products have not yet been ascertained.

### Polymerizable Erucic Acid Derivatives

Objective: To prepare new copolymers of vinyl behenate and dichlorobehenate with vinyl chloride and evaluate the properties of the polymers.

Accomplishments: Vinyl behenate and vinyl dichlorobehenate have been polymerized alone and with vinyl chloride and the polymeric products evaluated. Vinyl esters were prepared at NU, polymerized at the University of Arizona and evaluated at EU.

### Homo- and Copolymers of Vinyl Behenates

<u>Vinyl Esters of</u>	<u>Conversion, %</u>	<u>Softening Range ° C.</u>	<u>Intrinsic Viscosity</u>	
Technical behenic acid	83	62.5-63.5	0.24	
Dichlorobehenic	82	<25	0.15	
Copolymer (Vinyl Chloride				
Technical behenic	48-51	69-81	0.79	
Dichlorobehenic	48-80	53-77	0.75	
<u>Mechanic Properties</u>	<u>Ester, %</u>	<u>Elongation, %</u>	<u>Tensile (PRI)</u>	<u>Brittle Temp.° C.</u>
Copolymer behenate	23.5	10	470	28
"    dichloro-behenate	29.5	25	2,500	-2
"    stearate	30	170	2,800	6
Vinyl chloride plus 35% di- $\alpha$ -ethylhexyl phthalate		350	2,830	-30

This work can be summarized as follows: Vinyl behenate and its dichloro derivative will copolymerize with vinyl chloride. The dichloro behenate copolymer had superior low temperature properties but the behenate copolymer did not. Although the dichlorobehenate copolymer did not have a lower brittle point than the stearate copolymer, the stearate was superior in tensile strength, elongation, and in milling temperatures.



## G. NU ENGINEERING AND DEVELOPMENT PROCESS STUDIES ON NEW CROPS

### Introduction

During the year 1961, research was carried out by the engineering group on oriental mustard seed to develop a commercially feasible process for obtaining a high quality oil, a bland nutritious oil meal, and recovery of the byproduct and pungent factor, allyl isothiocyanate. A suitable process was developed which made use of integrated enzymatic hydrolysis to modify the thioglucoside to the volatile essential oil and separate this component by steam distillation. During seven weeks of trial runs, the process was tried out on a considerably larger scale with good success in the filtration extraction pilot plant of SU. This was followed by further improvements in the process in NU studies which produced better byproduct yields and improved protein quality.

During 1962, the mustard seed studies were completed and new studies were begun on the processing of Crambe abyssinica. Although Crambe contains far less allyl isothiocyanate product than mustard, it contains thioglucosides which upon hydrolysis release nonvolatile sulfur-containing compounds. A successful process for Crambe will involve developing an efficient oil extraction for the seed integrated with treatments to remove or inactivate the growth inhibitory substances present in the meal. In addition to detoxification of the meal, a second objective will be to obtain a high quality oil product reasonably free of sulfur compounds that can poison hydrogenation catalysts or possibly reduce functionality of the oil in industrial uses.

### Summary of Mustard Seed Process Development

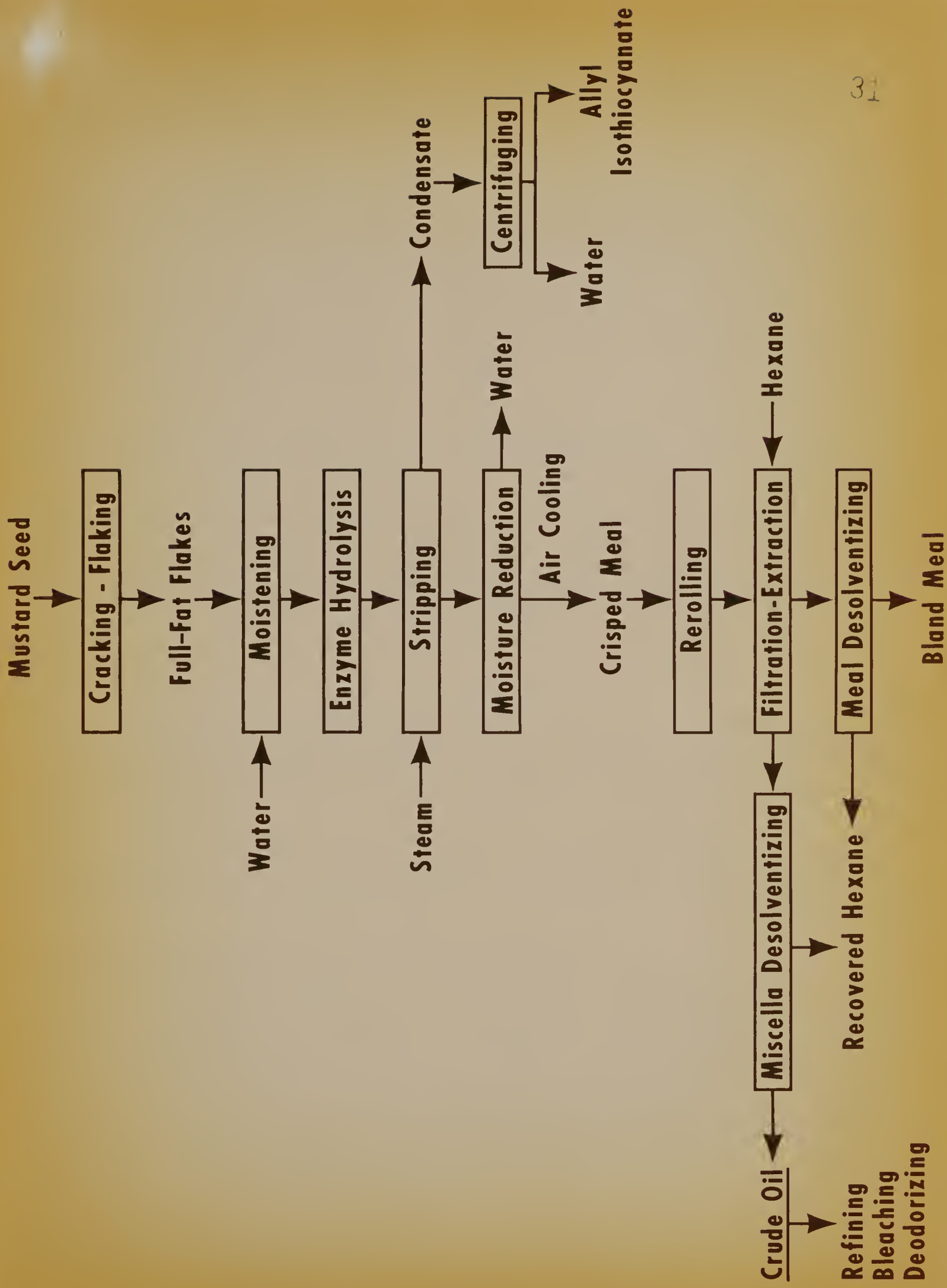
The new process for mustard seed involves removal of the glucoside in addition to the normal conversion of the seed into nonvolatile lipids and defatted oil meal. The pungent factor, allyl isothiocyanate, occurs naturally in mustard seed as the water-soluble thioglucoside, sinigrin. The isothiocyanate in the presence of moisture and heat is released from the glucoside by the enzyme "myrosinase" which occurs naturally in the seed. In the hydrolysis reaction, the sugar moiety of the thioglucoside is glucose and aglycone fraction is allyl isothiocyanate. The enzymatic process was successfully integrated into two commercial oil extraction processes, namely filtration-extraction and prepress-solvent extraction. The essential oil was separated and recovered during the cooking steps in both of these processes. Figure 1 shows a flow-sheet of the process using filtration-extraction.

### Meal Preparation

Studies were carried out to determine the feasibility of separating the hulls. A hull fraction could be separated by tempering, cracking in three stages, and screening with aspiration. This fraction, however, was high in lipid and protein, 15.5 percent and 22.4 percent, respectively. The hull-separated fraction represented about 15-20 percent of the seed weight, so that dehulling was considered to be uneconomical for a commercial process.

Optimum preparation of the solids for the ensuing process steps including solvent extraction was studied. Best results were obtained when the clean

# Flowsheet







whole seed was tempered to approximately 8 percent moisture and milled to thin flakes. Milling consisted of either cracking through corrugated rolls followed by flaking through smooth rolls, or by flaking the whole seed through smooth rolls directly.

### Separation of the Pungent Factor

Separation of the pungent essential oil allyl isothiocyanate, to eliminate its presence from the meal as well as to obtain good recovery of the pungent oil as a byproduct were important objectives. Optimum conversion of the thioglucoside depends on moisture content, temperature, and retention time. Conditions developed for the conversion were 30 percent moisture, 130° F., and a 15-minute retention period. The short retention time proved to be sufficient for complete conversion yet did not allow time for the essential oil to decompose or react with the protein.

Various techniques were tried for recovery of the essential oil from the converted meal. When the process was modified from simple distillation to atmospheric steam stripping, the recovery increased from 71.7 to 99.2 percent.

The recovered crude essential oil contained approximately 90 percent allyl isothiocyanate and 10 percent of an impurity previously unidentified. The unknown impurity was demonstrated to be allyl thiocyanate by gas-liquid chromatography. Since the thiocyanate isomerizes to isothiocyanate during atmospheric distillation, it appears possible that the isothiocyanate content of the essential oil can be increased.

### Protein Quality

Lysine, arginine, and histidine contents of mustard meal protein varied appreciably depending upon the degree of heat treatment during processing. Destruction of these amino acids was noted when the meals were dried excessively during cooking or when the defatted and desolventized meals were steam treated.

Where heating time intervals were held to a minimum and temperatures were maintained below 220° F., essentially no destruction of amino acids was obtained.

### Toxicological and Nutritional Evaluation of the Meal

Rat bioassays on the meals were carried out in cooperation with WU to evaluate mustard meal as a feedstuff. The program was initiated by studying the growth responses of rats to various levels of allyl isothiocyanate added to an addis basal diet. The basal diet consisted of 73 percent corn meal, 10 percent linseed meal, 10 percent crude casein, 3 percent cod liver oil, 2 percent alfalfa meal, 1.5 percent bone ash, and 0.5 percent sodium chloride. Thus, in such an addis diet, a surplus and a variety of protein is afforded so that the measured growth inhibition of the rats is caused by toxicity alone rather than amino acid deficiency.

Allyl isothiocyanate was fed at levels of 0.01-0.4 percent. Significant growth inhibition was not shown until the level reached 0.2 percent. However,



when the essential oil was added in combination with 20 percent mustard meal in the diet, significant growth inhibition was shown with only 0.05 percent allyl isothiocyanate. This indicates the possibility of a toxic-protein derivative of allyl isothiocyanate being formed.

Mustard meal by our process containing 0.00-0.01 percent allyl isothiocyanate, was fed at 20 percent level and 30 percent levels in combination with the addis basal diet. The meal fed at the 20 percent level showed good utilization with no significant growth inhibition. A similar growth response was obtained when soybean meal was fed at a 30 percent level. When the mustard level was increased to 30 percent, some growth inhibition was realized. Histopathological examination of thyroid tissues showed no abnormal growth effects for any of the mustard meals tested regardless of the level used.

In a second study, the mustard meal was fed as a 50 percent blend of mustard and soybean meal as the sole sources of protein in the diet. Growth responses of a 20-20 percent mustard-soybean meal were essentially equal to a 40 percent soybean control. Since the blended protein showed equal growth responses as a soybean basal diet alone, it appears that mustard meal as a supplementary source of protein will be satisfactory.

#### Progress in Crambe Process Development

Preliminary investigations on Crambe processing are well underway and considerable progress has been made. In these studies, it was shown that Crambe seed was adaptable to the filtration-extraction process. Initial milling studies showed that Crambe could be readily dehulled in conventional cracking rolls and vibrating screens with aspiration removal of the hull fraction. Meal protein was increased from 40 to over 50 percent by dehulling. The integrated enzymatic hydrolysis procedure as developed for the glucosides of mustard seed was applied to Crambe. Sulfur balances indicated that essentially complete enzymatic hydrolysis was attained in a 15-minute period. The thioxazolidone-type ring compounds formed in the hydrolysis were completely changed chemically so that the typical UV absorption disappeared during the moist cook preparation of the seed. The defatted meal produced by this process was fed to weanling rats at WU and found to be slightly growth inhibitory at a 15-percent feeding level and more pronounced inhibition was found at the 30-percent level. When the moist cook process was carried out on the meal after extraction of the oil, rat growth inhibition was very slight so that rat gain was equal to 94 percent of the control diet. Moist cooking after oil extraction, therefore, appears most promising. No thyroid enlargement, however, was found in any of the tests. This indicates that the ring compounds may be the only goitrogenic factor in Crambe.

#### Future Emphasis

Crambe has much potential based on the very promising crop yields and on the structural characteristics of its oil. However, like rapeseed, the seed contains nonvolatile cyclic growth inhibitory compounds which need to be removed or inactivated by processing. If Crambe is to develop as a successful crop in the U. S., an economic process must be found for separating the seed into pure oil and nontoxic meal products. The oil should be relatively free

of sulfur contamination so that after refining and bleaching, hydrogenation can easily be carried out without catalyst poisoning effects. The oil meal must be treated so as to yield a product which is palatable, nutritional, and competitive with other commercial oilseed meals.

Our present knowledge indicates that the toxic or growth inhibitory effects in Crambe meals may be due to thiooxazolidone-type ring compounds, non-volatile hydrolysis products of thioglucosides, or in lesser degree, the precursor thioglucosides themselves. Basic chemical and previous engineering information will be used to develop processes for Crambe. These will be based on treatments both before and after lipid separation. In the detoxification studies basic approaches will include, (1) integrated enzymatic hydrolysis and (2) enzyme deactivation with no hydrolysis of thioglucosides. Solvent extraction of residual thioglucosides from defatted meals will also be studied. Meals prepared by several experimental processes will be sent to WU for toxicological and pathological evaluation by rat feeding tests. Large quantities of Crambe will be processed to produce samples of crude, refined and bleached, and refined, bleached and deodorized oils for sample evaluation by industrial firms. When promising results are shown in these tests, the feeding studies will be expanded to larger animals such as poultry, sheep, or possibly cattle.



Publications in Print

Mustard Seed Processing: Bland Protein Meal, Bland Oil, and Allyl Isothiocyanate as a Byproduct, G. C. Mustakas, L. D. Kirk, and E. L. Griffin, Jr., J. Am. Oil Chemists' Soc., 39 (8) 372, 1962.

Submitted for Publication

Pilot-Plant Application of Filtration-Extraction to Oriental Mustard Seed, G. C. Mustakas, E. L. Griffin, Jr., E. A. Gastrock, E. L. D'Aquin, and E. L. Patton.

Submitted to Journal of Agricultural and Food Chemistry.

In Preparation

Mustard Seed Processing: Improved Methods for Isolating the Pungent Factor and Controlling Protein Quality, G. C. Mustakas, L. D. Kirk, and E. L. Griffin, Jr.

For publication in J. Am. Oil Chemists' Society.

Effect of Heat Treatment on Mustard Protein: Evidence of Non-Enzymatic Browning. J. E. McGhee, L. D. Kirk, and G. C. Mustakas. For publication in J. Am. Oil Chemists' Society.

Presentations

Mustard Seed Processing to Yield Bland Protein Feed Meal and Oil with Recovery of Allyl Isothiocyanate. G. C. Mustakas, L. D. Kirk, and E. L. Griffin, Jr. Presented at National A.O.C.S. meeting, St. Louis, Mo., May 1-3, 1961.

Pilot-Plant Application of Filtration Extraction to Oriental Mustard Seed. G. C. Mustakas, L. D. Kirk, and E. L. Griffin, Jr. Presented at National A.C.S. meeting, Washington, D. C., March 20-29, 1962.

Mustard Seed Processing: Improved Methods for Isolating the Pungent Factor and Controlling Protein Quality. G. C. Mustakas, L. D. Kirk, and E. L. Griffin, Jr. Presented at International A.O.C.S. meeting, Toronto, Canada. October 1-4, 1962.

Patents Filed

Method of Obtaining Detoxified Mustard Seed Products. G. C. Mustakas and L. D. Kirk.

Method of Obtaining Detoxified Meal from Seeds Containing both Isothiocyanate and Thiioxazolidone. G. C. Mustakas and L. D. Kirk.

#### H. Thioglucosides of selected new oilseeds other than crambe

Estimation of the oxazolidinethione and volatile isothiocyanate formed enzymatically from thioglucosides present, was continued. From the Cruciferae 23 new accessions were analyzed, 11 species of which no previous literature information was found. For the 23 accessions the volatile isothiocyanate ranged from 0.0 to 18.8 mg. per g. of meal, and the oxazolidinethione from 0.0 to 7.5 mg. per g. of meal. Of these species the 4 which had very low or no volatile isothiocyanate or oxazolidinethione were unfortunately also not high in erucic acid content.

Ten species of the *Limnanthes* genus of the Limnanthaceae family, only one for which prior information was available, were analyzed. Only one species (*Limnanthes alba*) contained oxazolidinethione as 2.6 mg. per g. of meal. All the species contained volatile isothiocyanates ranging from 7.7 to 11.3 mg. per g. of meal. Paper chromatography of the thiourea derivatives of the volatile isothiocyanates indicated all the volatile isothiocyanates from this genus to be of an aromatic type.





# **Progress Report EU NEW CROPS RESEARCH**

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**JANUARY 25, 1963**

**ARS New Crops Meeting  
Washington, D.C.**





Summary Statement

Larger-scale extractions have been made with Vernonia anthelmintica seed to learn the effects of stepping up small-scale experiments and to obtain low free fatty acid oil for distribution. At present we are unable to supply Vernonia oil to industrial agencies requesting material for evaluation. To date we have distributed Vernonia products to 17 industrial and 10 institutional organizations for research studies but have a long waiting list of requests. Several large manufacturers report enthusiastically on their preliminary evaluation of the use of Vernonia oil in plastic formulations; they have requested additional oil in 1-5 gallon quantities.

Report

To other agencies, for evaluation studies, we have distributed Vernonia products as follows: 75 pounds of seed for extraction purposes, 52 pounds of seed marc, 45 pounds of seed oil, 2 pounds of trivernolin, 1 pound of vernolic acid and 1/4 pound of 1,3-divernolin. To fill our own experimental needs we have prepared and consumed about 50 pounds of oil to date.

We have recently installed a Laukoff rolling mill for flaking Vernonia seed and have equipped it with an automatic feed so that we can roll seed at as high a rate as 2 pounds per minute. We have found that flaked seed extracts more efficiently than ground Vernonia seed. On pilot plant extractions of about 60-pound batches of seed, we have not as yet been able to prepare trivernolin-rich oils with quite as low a free fatty acid content as those prepared on the laboratory bench, either by the use of the rapid technique (extraction immediately following flaking) or by autoclaving (to kill enzyme activity) of the seed prior to flaking or grinding. On the bench we obtained, by the rapid extraction technique, oils with

a FFA (free fatty acid) content of less than 2%; in the pilot plant 3.4% represents our best effort. In the laboratory by autoclaving seed prior to extraction we have prepared oils with a FFA content of less than 0.5% but in the pilot plant this figure has not been lower than 1.3%. Plans call for further pilot plant experimentation to find out how to obtain lower free fatty acid values. If the seed were flaked in the solvent followed by rapid removal of the latter it seems likely that the prepared oil's FFA content would be lower.

The domestic seed (Nebraska-grown in 1961) appears to be more difficult to extract than that of Indian or Pakistanian origin and there are some differences in seed composition. The reasons for these differences are not apparent at this writing. It is of primary importance that we make comparable composition studies on the domestic seed oil to those made on the imported seed oil.

We have succeeded in preparing chromatographically (TLC) pure vernolic acid, trivernolin and 1,3-divernolin. Silicic acid column chromatography alone will not purify trivernolin and 1,3-divernolin since they each contain an impurity which moves at the same rate at which they migrate. TLC shows base-line immovable impurity also present in all three compounds but this is readily removed by column chromatography.

## II. EVALUATION OF VERNONIA PRODUCTS IN PLASTIC FORMULATIONS

### Summary Statement

Studies on metal salts of epoxyoleic acid as heat and light stabilizers of plasticized poly(vinyl chloride) have been continued. The results showed that these salts were superior to the stabilizers presently used. The



combination of calcium and zinc salts which can be used as "food grade" stabilizers showed exceptional stabilizing qualities when compared with a commercial stabilizer.

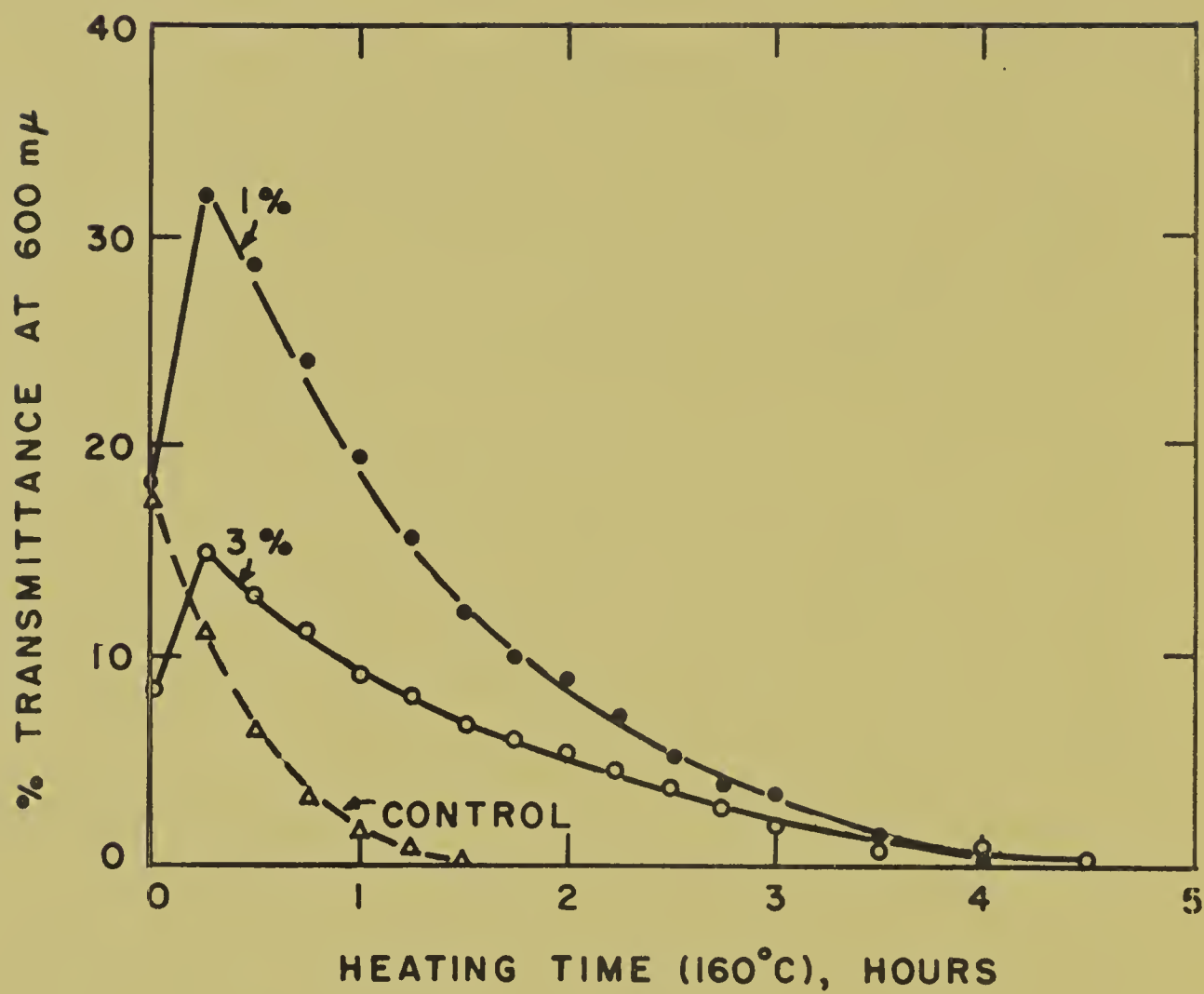
### Report

Evaluation of vernolic acid as a stabilizer of poly(vinyl chloride) was made at the 1 and 3% levels. Light amber, transparent, molded sheets were obtained. Zinc and calcium salts and mixtures of the salts were also evaluated as "food grade" stabilizers. The zinc salt produced no stability effect on the molded sheets at either the 1 or 3% levels yielding black sheets. At the 1% level the calcium salt (Fig. 1) produced a transparent amber colored sheet; at the 3% level a dark amber semi-transparent sheet. The mixed calcium-zinc salts produced sheets ranging in color from a very faint yellow to amber; the higher stabilizer level produced a more opaque sheet.

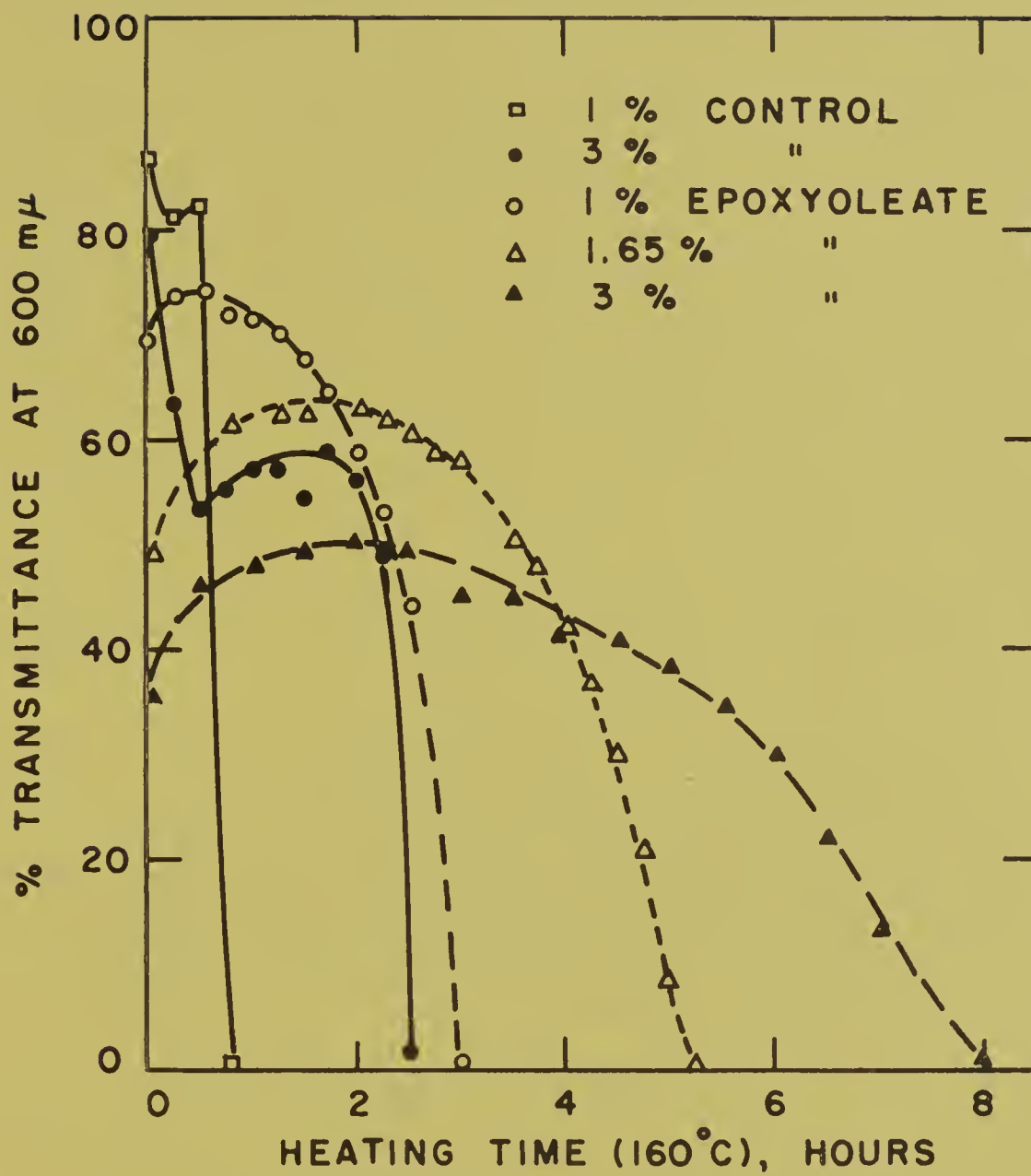
Vernolic acid showed a certain degree of stabilizing ability at both levels with the higher level producing the greatest heat stability. The calcium salts exhibit good heat stability; the greatest heat stability was at the lower level (1%). However, this was somewhat less than the stability previously reported with the barium salt of the acid.

Mixtures of calcium and zinc (Fig. 2) showed that a 5:1 ratio of calcium to zinc produced the greatest heat stability. These were compared with a commercial calcium-zinc stabilizer. At all levels greater heat stability was shown by the calcium-zinc combination than with the commercial stabilizer at the same levels. The commercial stabilizers produced a dry waxy exudate after heat treatment which was not present in the vernolic samples. The combination of calcium and zinc salts produced a marked synergistic effect.

FIG. 1  
CALCIUM EPOXYOLEATE



## 5:1 RATIO Ca:Zn SALT





The light stability of vernolic acid at the 1% level was found to be as good or better than some salts or mixtures of barium and cadmium salts.

The calcium salt at the 1% level was found to be a more effective light stabilizer than other salts (barium and cadmium). At the 3% level the calcium salt was almost as effective as other barium or cadmium salts. The mixed calcium and zinc salts produced exceptional light stability when compared with the commercial calcium-zinc stabilizer. The salts showed failure after 360 hours' exposure compared with 768 hours for the vernolic salt mixture.

### III. ENZYME STUDIES ON VERNONIA ANTHELMINTICA (L.) WILLD. SEED

#### Summary Statement

We have discovered that under proper handling conditions of ground Vernonia seed, both hydrolytic and hydrating enzymatic action occurs. The result of both is the production of an oil rich in (+) threo-12,13-dihydroxyoleic acid. We believe that we have obtained data which demonstrated that both the hydrolytic and hydrating principles are enzymatic, not chemical or bacterial.

#### Report

Following previous studies which were concerned with inhibiting the lipase enzyme activity in Vernonia seed, in order to prepare oil rich in trivernolin, research was initiated to learn if the lipase activity could be accelerated for the purpose of producing vernolic acid as a major product. Freshly ground seed incubated in an air atmosphere became moldy so incubation was carried out under nitrogen. Oils prepared from freshly ground seed incubated in a nitrogen atmosphere saturated with water-vapor gave the expected conversion of glycerides to free fatty acids. Some unexpected changes also were encountered: increase in iodine number, decrease in

oxirane oxygen percentage and apparent solubility changes. In last year's report we mentioned this but were unable to explain these results. Further researches have disclosed the cause of these alterations: an enzyme system is present which under the conditions of the experiment is capable of hydrating the (+)-D-epoxyoleic acid present in the seed to (+) threo-12,13-dihydroxyoleic acid. Highly interesting also is the fact that by chemical methods such as acetolysis of the seed oil the racemate is readily prepared from which the (-) isomer can be separated by repeated fractional crystallization.

(+)-threo-12,13-Dihydroxyoleic acid was isolated from mature seed that had been incubated under nitrogen in a water saturated atmosphere. The yield of this compound was increased approximately three and one-half times (about 32% of the weight of oil extracted) by incubating the seed with twice its weight of water. In contrast, none of the dihydroxy compound was found when seed was autoclaved to inactivate the enzyme systems before it was incubated with twice its weight of water. In fact, TLC showed that the oil obtained from this enzyme inactivated seed contained mainly trivernolin and normal triglycerides; the chromatogram of this oil was identical with that of a normal (low FFA) Vernonia oil.

#### IV. ANALYTICAL STUDIES ON VERNONIA

##### Summary Statement

An improved method has been devised for the analysis of epoxy fatty acid containing oils. In the determination of the fatty acid composition of Vernonia oil the values obtained for the epoxy components agreed favorably with those obtained by oxirane oxygen procedures.

### Report

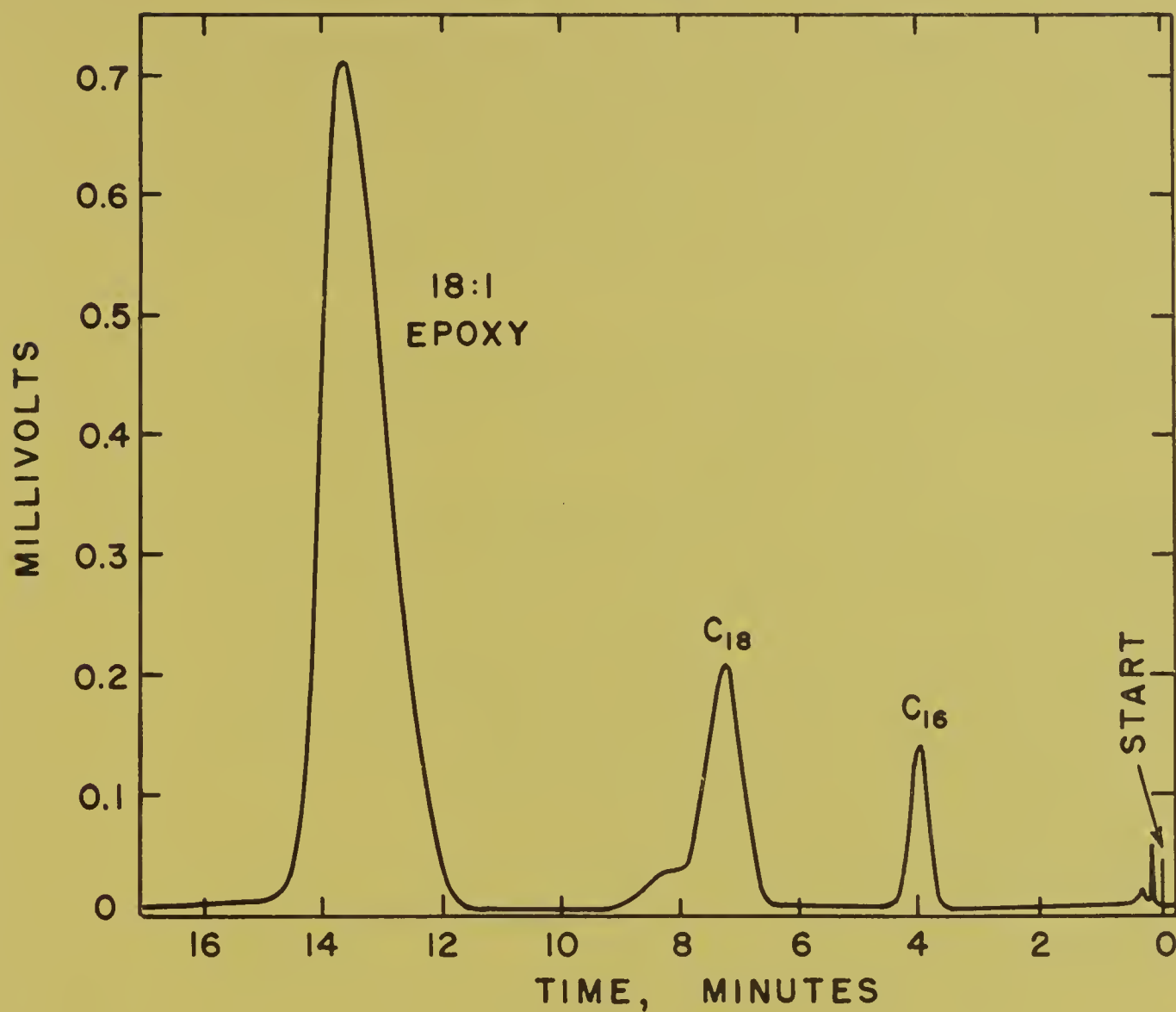
Analysis of fatty acid composition of Vernonia seed oil by gas-liquid chromatography (GLC) of the methyl esters on polyester columns was discussed in the preceding report. Alteration of the methyl epoxyoleate during the analysis was evident but could be corrected for by use of internal standard and calibration factors.

Further study of GLC analysis of epoxy oleic acid containing oils has resulted in an improved method. Columns packed with 15% silicone rubber (SE-30) on Chromosorb W showed little or no evidence of alteration of epoxyoleate during GLC analysis (Fig. 3). Analyses based on peak area measurements on chromatograms of known mixtures containing pure epoxyoleate were in excellent agreement with the known values. Similarly GLC analysis of epoxyoleic content of Vernonia oils gave values in close agreement with those by oxirane determination. GLC on silicone rubber stationary phase, however, does not separate saturated and unsaturated esters of the same chain length, such as methyl stearate, oleate, and linoleate. Therefore, if the respective percentage of these in Vernonia oil are also desired, a second column containing a polyester stationary phase should be employed to give the relative peak areas of these individual components. These ratios related to their combined area percentage determined on the silicone column gives the percentage of each component.

There are distinct advantages in the use of this silicone column for the analysis of epoxy fatty acid containing oils over the use of the polyester column with correction factors as described previously.



FIG. 3  
G.L.C. OF VERNONIA ANTHELMINTICA OIL METHYL ESTERS



In addition to being generally more reliable, less time is required particularly in the calculation of the percentage of each component. The improved method is also more widely adaptable to other oxygenated fatty acid compositions. Preliminary results indicate that it may also be suitable for di-epoxy fatty acid esters some of which occur in commercially epoxidized oils.

## V. STABILITY STUDIES ON VERNONIA OIL AND TRIVERNOLIN

### Summary Statement

Planned stability tests on V. anthelmintica seed oil and on trivernolin have been initiated. The seed has been stored at room temperatures for 1 year exposed to air without change in composition of its oil. The oil has been found stable after 2-1/2 years' storage under nitrogen in the dark at 5°C.; however, oil stored at 100°C. has shown some loss in epoxy fatty acid content in 10 days. Trivernolin stored for 3 months under nitrogen (or protected with an antioxidant) has shown no change in composition when stored at 0° or at room temperatures; it has shown "epoxy" loss, however, at 100° under atmospheric conditions in 10 days.

### Report

Samples of low free fatty acid oil and of trivernolin were prepared for stability tests as follows: (1) No stabilizer added, exposed to atmospheric conditions; (2) 0.3% tertiary butyl hydroquinine added; and (3) no stabilizer added, stored under N<sub>2</sub> atmosphere. These samples were stored under the following conditions: (1) Room temperature, protected from light; (2) room temperature, exposed to light; (3) 100°C.; and (4) 0°C. The samples were checked at various intervals to determine the effects of storage on the FFA content, oxirane oxygen percentage and on their iodine value.

These samples have now been stored over 2500 hours. The only appreciable changes, in either the low FFA Vernonia oil or the trivernolin, occurred in the samples stored at 100°C. These changes, notably a loss of epoxy, began at approximately 200 hours. Storage under nitrogen gave more protection than addition of the stabilizer.

#### VI. PUBLICATIONS

1. "Vernonia anthelmintica (L.) Willd. Seed Oil and Salts of Vernolic Acid as Stabilizers for Plasticized Poly(Vinyl Chloride)," G. R. Riser, J. J. Hunter, J. S. Ard and L. P. Witnauer. J. Am. Oil Chemists' Soc., 39, 266-268 (1962).
2. "Vernonia anthelmintica (L.) Willd. Trivernolin, Divernolin and Vernolic Acid from the Seed Oil," C. F. Krewson, J. S. Ard, and R. W. Riemenschneider. J. Am. Oil Chemists' Soc., 39, 334-340 (1962).
3. "Vernonia anthelmintica (L.) Willd. (+)- and (-)-Threo-12,13-Dihydroxyoleic Acid," W. E. Scott, C. F. Krewson, and R. W. Riemenschneider. Chemistry and Industry (in press).

#### VII. PRESENTATIONS

1. "Relationship between Structure of C<sub>18</sub> Acids and Their Salts and Effectiveness in Poly(Vinyl Chloride) Stabilization," G. R. Riser, J. J. Hunter, J. S. Ard and L. P. Witnauer. Presented at the 53rd Annual Meeting of the American Oil Chemists' Society, New Orleans, La., May 7-9 (1962).
2. "The Transmethylation of Vernonia anthelmintica Seed Oil and the Isolation of Methyl Epoxyoleate," R. A. Barford, S. F. Herb, F. E. Luddy, P. Magidman and R. W. Riemenschneider. Ibid.
3. "Vernonia anthelmintica (L.) Willd. Oxygenated Lipid Components in the Seed," C. F. Krewson. Presented at the 142nd National Meeting of the American Chemical Society, Atlantic City, N.J., Sept. 9-14, 1962).
4. "Vernonia anthelmintica (L.) Willd. Enzymatic Studies. Production of (+)-threo-12,13-Dihydroxyoleic Acid," W. E. Scott, C. F. Krewson, and R. W. Riemenschneider. Presented at the 36th Fall Meeting of the American Oil Chemists' Soc., Toronto, Canada, October 1-4, 1962.

#### VIII. PATENTS

1. "Preparation of (+)-threo-12,13-Dihydroxyoleic Acid," W. E. Scott, C. F. Krewson and R. W. Riemenschneider. Patent application filed.





UNITED STATES DEPARTMENT OF AGRICULTURE

AGRICULTURAL RESEARCH SERVICE

REPORT  
ON  
NEW CROPS RESEARCH

BY

SOUTHERN UTILIZATION RESEARCH AND DEVELOPMENT DIVISION  
NEW ORLEANS, LOUISIANA

JANUARY 1963





UNITED STATES DEPARTMENT OF AGRICULTURE  
AGRICULTURAL RESEARCH SERVICE

REPORT OF  
SOUTHERN UTILIZATION RESEARCH AND DEVELOPMENT DIVISION  
ON  
NEW CROPS RESEARCH  
JANUARY 1963

During 1962 development research was carried out at the Southern Utilization Research and Development Division on oils from Limnanthes Douglasii; on capric acid, the principal acid component of oil from Cuphea llavea and Cuphea ignea; and oils containing petroselinic acid as the principal fatty acid. Petroselinic acid-bearing oils, primarily from Umbelliferae, were again introduced into the program as a result of the report last year from Crops Research that fennel had self-seeded or come up from root stock a second year in a Missouri planting and produced a reasonably good yield of seed.

Oil from Limnanthes Douglasii, Meadow-foam

Separation of component fatty acids. An adequate supply of oil has been obtained by a pilot-plant extraction of 100 pounds of Limnanthes Douglasii seed. This extraction yielded 22.4% of oil.

A number of different procedures were investigated to determine their suitability for separation of the principal fatty acids of Limnanthes Douglasii by either chain length or degree of unsaturation. As has been previously reported, these fatty acids are:

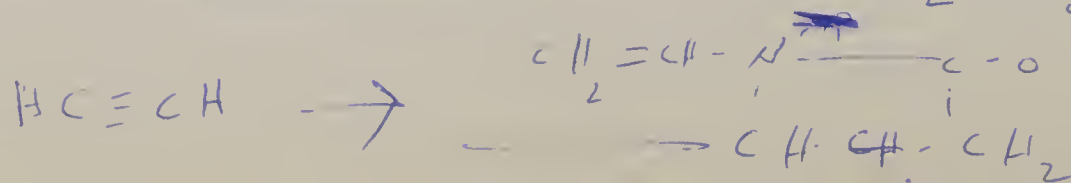
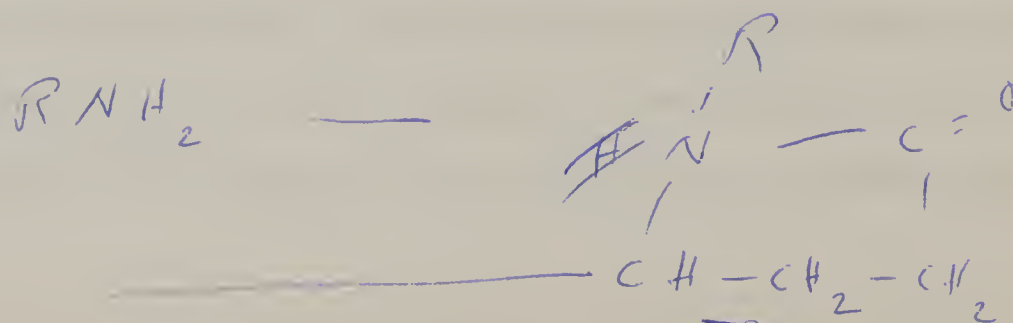
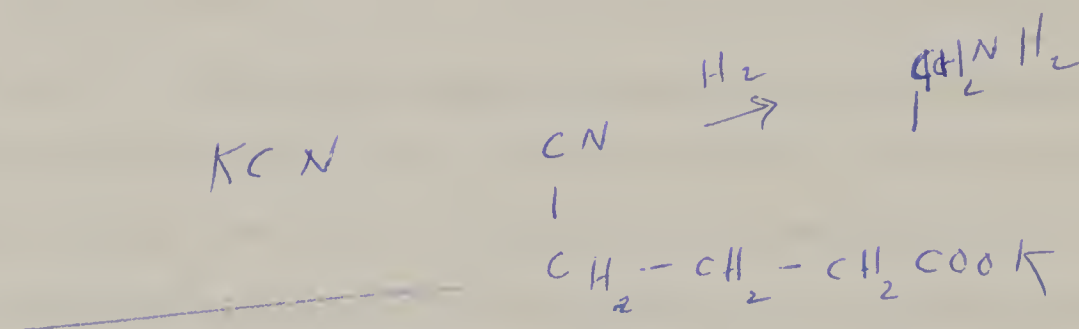
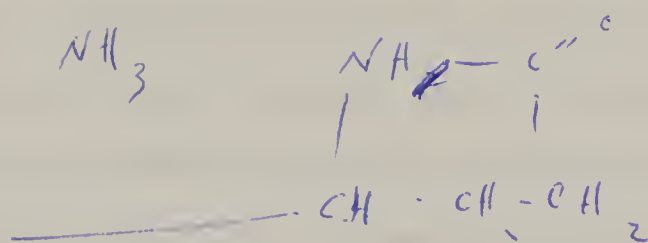
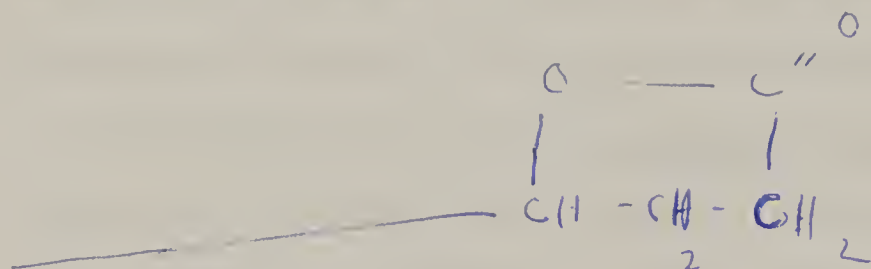


<u>cis</u> -5-eicosenoic acid	65%
<u>cis</u> -5-docosenoic acid	7%
<u>cis</u> -13-docosenoic acid	13%
<u>cis</u> -5- <u>cis</u> -13-docosadienoic acid	10%

Neither urea complex precipitation of Limnanthes Douglasii fatty acids nor fractional crystallization from a variety of solvents of the acids, esters, mercuric acetate addition product of the esters or the potassium salts derived from Limnanthes Douglasii oil resulted in satisfactory yields of the major components. Fractional distillation of the methyl esters resulted in a good yield of methyl eicosenoate, but the yield of C<sub>22</sub> esters was poor and all fractions contained some trans bonds, while the unsaturation in the original esters was wholly cis. Further studies are being made to determine whether conditions can be established for separating the esters by distillation without their deterioration. A preliminary investigation of the feasibility of using preparative gas-liquid chromatography for purification of the various components is also underway.

Lactonization of Limnanthes Douglasii fatty acids. During an investigation of "Positional Isomerism in the Formoxylation of Petroselinic Acid," L. L. Placek and W. G. Bickford (J. Org. Chem. 26, 864 (1961)) observed migration of the double bond of petroselinic acid toward the carboxyl group in the presence of perchloric acid as a catalyst. The migration progressed no further than the 5-carbon position. They postulated "It is possible that a carbonium ion in the 4-carbon position may have been internally stabilized by the formation of the lactone of





4-hydroxyoctadecanoic acid." This interesting observation together with a suggestion of Dr. W. E. Hanford, Vice President for Research and Development, Olin Mathieson Chemical Corporation, New York, New York, during a visit to SU on March 13, 1962, led us to consider lactonization as a promising approach to the chemical modification of long-chain monounsaturated fatty acids having double bonds closer to the carboxyl group than does oleic acid. The lactonization reaction was also discussed with Dr. Daniel Swern of EU, who had underway some research in this field. This was subsequently presented in the Fall Meeting of the American Oil Chemists' Society, Toronto, Canada, October 1-4, 1962 (J. S. Showell, W. R. Noble and Daniel Swern, "Preparation of gamma-Stearolactone by Perchloric Acid Isolation of Oleic Acid"). Subsequent to his visit, Dr. Swern reported to us that petroselinic acid in their hands gave no higher yields of the gamma-stearolactone than did oleic acid.

Gamma-lactones derivable from Limnanthes Douglasii monoenes appear to be promising starting materials for a variety of chemicals having potential industrial utility. Accordingly, an investigation of the acid catalyzed lactonization of Limnanthes Douglasii fatty acids has been undertaken. Of a large number of acids of both the classical and Lewis types which have been investigated for this purpose, 70% perchloric acid was the most effective, giving ca. 80% conversion of 5-eicosenoic acid to the lactone while the next most effective catalyst, p-toluenesulfonic acid, gave ca. 65% conversion. However, because of the hazards involved in

Surface active agents, ore flotation  
agents, corrosion inhibitors



handling large quantities of perchloric acid, the feasibility of improving the economics of the p-toluenesulfonic acid catalyzed reaction by recycling the catalyst and unreacted fatty acid is being studied.

#### Cuphea and Capric Acid

Capric acid and some of its derivatives have been obtained from commercial sources since Cuphea is not well adapted to seed collection from the ornamental plants available and since for most purposes, oil of the seed would have an advantage only where direct uses are to be developed for the triglyceride itself. It has been called to our attention that a triglyceride high in fatty acids of 8- and 10-carbon atoms might have interesting pharmaceutical applications for patients suffering with chyluria. Mr. Vigen K. Babayan, Director of Research and Development, Drew Chemical Corporation, reported to Miss Audrey T. Gros at the Fall Meeting of the American Oil Chemists' Society that they have been cooperating with the Institute of Nutrition Sciences, Columbia University, in some clinical experiments. They have found that patients suffering with chyluria can be relieved with formula diets containing medium chain triglycerides, composed principally of  $C_8$  and  $C_{10}$  fatty acids and prepared synthetically. There was also a decrease in proteinuria and hematuria which normally accompany the condition. Although results of these investigations have not been published, they have been presented before a meeting of the American Society for Clinical Nutrition. Studies are being continued. Dr. Babayan inquired concerning the USDA program of screening new oilseed crops. The above work indicates a definite use for an oilseed containing  $C_8$  and  $C_{10}$  fatty acids. Perhaps Cuphea could supply this need.



Survey of literature on decanoic acid. Since much work has been done on decanoic acid, it was necessary to search the literature for what had been done before starting out to try to find new uses for it. More than 2000 references to decanoic acid or its derivatives were found in chemical abstracts. All of these abstracts were read, about 1500 of them copied onto McBee Keysort cards by a Xerox copying machine, and about 800 of the more interesting references coded by punching the holes around the periphery of the Keysort cards. The code index contains about 1200 derivatives of decanoic acid, more than 50 suggested uses, as well as methods of preparation, physical properties, etc. The most frequently mentioned uses in the literature are: soaps and other surface-active compounds, plasticizers, and pesticides. A selective survey of the literature on decanoic acid is being prepared.

Decanoic acid esters of starch. Modification of starch with decanoic acid for adhesives was suggested as a possible use for the acid (1). Esters were prepared either by adding the acid chloride to the starch dispersed in a pyridine-dioxane solvent and heating (2), or by dispersing in a 60% pyridine in water solution, azeotropically distilling the water off, then heating the pyridine dispersion of the starch with decanoic anhydride (3). Esters of corn starch (Fisher Scientific Company, or commercial corn pearl starch) were prepared containing 2.6, 15.8, 24.7, 41.5, 56.9 and 72.7% decanoyl. Theoretically, a completely esterified starch would contain 74.5% decanoyl assuming the starch to be composed entirely of glucopyranose units and that three hydroxyls in each group are available for esterification.





The differently treated samples of starch were compared with the untreated starch by making them into pastes with water where possible and testing their strength as adhesives. Usually the pastes were made by heating with stirring 5 g. of starch or ester to 60 cc. water (4). The strengths as adhesives were measured by gluing together strips of cotton duck cloth one inch wide, drying and measuring the peel strength in pounds per linear inch required to peel one strip from the other on the Instron machine (5).

None of the esterified starches would paste by heating with water, as would the original starch. Some of the partially esterified starches dissolved enough in organic solvents to give slight adhesion but the adhesive strength was less than that of the untreated starch pasted with water.

The completely esterified starch (72.7% decanoyl) was soluble in hexane and other non-polar solvents. Application of a 32% solution of the ester in hexane as an adhesive gave a peel strength of 1.54 pounds per inch as compared to 2.40 pounds per inch for the untreated starch pasted with water. The adhesive from the ester was somewhat more resistant to moisture than that prepared with water from the untreated starch.

To determine whether the swelling of the starch in pyridine had any effect on the adhesive strength of the starch, starch was swollen in pyridine, given the same heat treatment as in preparing the esters, then recovered. The peel strength of adhesive made from the recovered starch was as high as that made from the untreated starch.





The decanoic acid starch esters were heated to see if they would soften and could be applied as hot adhesives. None of the partially esterified samples softened without charring, but the completely esterified starch softened at about  $136^{\circ}$  with slight darkening in color and an offensive odor while hot. In the molten state it was very stringy and sticky and stuck to anything contacted with it; wood, glass, metal, paper. Quantitative results could not be obtained on the peel strength of cotton duck strips glued together with it because of difficulty in getting uniform application. Non-porous materials seemingly were glued together very strongly with it and after six months in the laboratory adhesion seems as strong as ever. The cold ester is not sticky to the touch but is not brittle.

#### References

1. Report on New Crops Research, SURDD, January, 1962, p. 10.
2. Audrey T. Gros, and R. O. Feuge. J. Am. Oil Chemists' Soc., 39, 19-24 (1962).
3. James W. Mullen, and Eugene Pacsu. Ind. and Eng. Chem. 34, 1209-1217 (1942).
4. Roger Dulac. Industrial Cold Adhesives. Charles Griffin and Co., Ltd. London, 1943. pp. 34-35.
5. ASTM Designation; D-903-49.

Highly esterified starch, might find application  
as a solvent soluble, dip or spray type adhesive



### Petroselinic Acid

Although work on Limnanthes and Cuphea seed oils was assigned to the Southern Division in 1961 in place of the work on petroselinic acid-containing oils, the work on petroselinic acid was reactivated because a self-seeding crop of fennel had shown so much promise for commercial production of the seed.

A paper was presented at the Spring Meeting of the American Oil Chemists' Society on the preparation of dodecylamine and 6-aminohexanoic acid from petroselinic acid.

Literature survey. A review of petroselinic acid and its derivatives was completed and presented at the 142nd National Meeting of the American Chemical Society, Atlantic City, New Jersey, September 9-14, 1962, by Miss Lida L. Placek. This paper has been submitted to the Journal of the American Oil Chemists' Society for their consideration for publication. If it is not considered suitable, it will be released as an ARS publication.

Analysis of fennel seed oil for petroselinic acid. Dr. J. R. Haun, New Crops Research Branch, Beltsville, Maryland, sent us nine samples of fennel seed to be analyzed for oil and for petroselinic acid. Since there was no accepted method for determining petroselinic acid quantitatively in the presence of oleic acid, we have estimated it by ozonizing and oxidizing the mixed acids, determining the amount of adipic acid by titrating eluate fractions from a silicic acid column and calculating the amount of petroselinic acid from the amount of adipic acid obtained. Determinations of adipic acid from pure petroselinic acid under the





conditions used gave a recovery of 81% of theory. Consequently, the amount of adipic acid recovered from the mixed acids was multiplied by 100/81 before calculating it to an equivalent weight of petroselinic acid. The oil content of the fennel seeds calculated to a dry basis and the petroselinic acid content of the oil are given in Table I.

Table I

## Analysis of Fennel Seed for Oil and Petroselinic Acid

Sample No.	Ca. Seed 1/A field	Moist. in seeds, 1/ %	Oil in seed dry basis 2/ %	UNAP, %	Petroselinic acid in oil 3/ %	PA mixed acid, %
1	1st planting 4-20-61 12" spacing	10.7	19.0	11.2	59.7	76.1
2	1st planting 4-20-61 24" spacing	11.0	17.7	11.3	57.9	70.9
3	1st planting 4-20-61 36" spacing	10.7	16.3	13.5	62.1	77.6
4	2nd planting 5-10-61 12" spacing	10.0	17.2	12.1	53.6	66.0
5	2nd planting 5-10-61 24" spacing	10.2	17.8	11.9	55.0	66.4
6	2nd planting 5-10-61 36" spacing	10.5	15.6	12.7	58.7	70.6
7	3rd planting 5-23-61 12" spacing	10.5	11.6	—	59.2	77.1
8	3rd planting 5-23-61 24" spacing	10.2	11.1	15.2	59.8	77.5
9	3rd planting 5-23-61 36" spacing	10.9	10.7	15.3	46.8	60.9

1/ American Oil Chemists' Society Method Aa 4-38.

2/ American Oil Chemists' Society Method Ad 5-52, C(b).

3/ Mixed acids ozonized and oxidized with hydrogen peroxide. Adipic acid was determined by chromatography on silicic acid and the results multiplied by 1.2292 before calculating to petroselinic acid.





The methyl esters of the mixed fatty acids from the fennel seed oil and also of the ozonized and oxidized mixed acids are being analyzed by GLC.

#### Trimethylol Derivatives of Aldehydes Derivable from New Crops Oils

Trimethylol derivatives of octanal, nonal, decanal, and dodecanal have been prepared and characterized. These were prepared by reacting the aldehyde with an excess of formaldehyde and base as a preliminary step to preparation of trimethylol derivatives of dodecanal and adipaldehydic acid obtained by the ozonization and reduction of petroselinic acid. Yields have been low, the highest being about 38% of the theoretical weight of trimethylol heptane from octanal. This compares with a reported yield of about 50% in laboratory preparation of pentaerythritol from acetaldehyde, a reaction carried out on a large scale commercially. (See Organic Syntheses, Coll. Vol. I, pp. 425-7.)

#### Antimicrobial Activity of Some Fatty Acid Derivatives

New derivatives of decanoic acid or its homologs. Propargyl 2-bromodecanoate, 4-(2-bromodecanoyl)morpholide, 4-(2-decenoyl)morpholide along with the 2-octenoyl-, 2-nonenoyl-, and 2-dodecenoyl-homologs of the latter have been prepared and tested as antimicrobial agents by Dr. A. F. Novak at Louisiana State University. All of these compounds were effective against a wide range of microorganisms and since they are new compounds a patent is being prepared claiming their use as antimicrobial agents. They are also being offered to the National Institutes of Health for testing as anticancer agents.



Petroselinic and decanoic acid derivatives. The following fatty acid derivatives have been screened for antimicrobial activity against gram positive and gram negative bacteria and 22 pathogenic fungi in cooperative research with Dr. A. F. Novak.

6-Hydroxycaproic acid

4-(6-Hydroxycaproyl)morpholine

4-(6-Hydroxycaproyl)-2,6-dimethylmorpholine

Lactone of 6-hydroxycaproic acid

Ethyl 6-hydroxycaproate

Sodium 6-hydroxycaproate

6-Aminocaproic acid

Lactam of 6-aminocaproic acid

Decanoic acid

4-Decanoylmorpholine

4-Decanoyl-2,6-dimethylmorpholine

N-Butyldecanamide

N-Isoamyldecanamide

N-Cyclohexyldecanamide

N,N-Bis(2-decanoyloxyethyl)decanamide

N-Decanoylpiperidine

N-Decanoylhexamethylenimine

N-Decanoyl-N'-methylpiperazine

N,N-Didecanoylpiperazine

N-Decanoyltetrahydroquinoline

Lauric acid





4-Lauroylmorpholine

N,N-Bis(2-hydroxyethyl)lauramide

N,N-Bis(2-decanoyloxyethyl)lauramide

N,N-Bis(2-lauroyloxyethyl)lauramide

6(7)-Hydroxystearic acid

4-(6(7)-Hydroxystearoyl)morpholine

6,7-Epoxy stearic acid

Petroselinic acid

4-Petroselinoylmorpholine

Methyl petroselinate

Parsley seed acids

Morpholides of parsley seed oil fatty acids

Morpholides of rapeseed acids

N,N-Bis(2-acetoxyethyl)amides of rapeseed acids

Petroselinylamine hydrochloride

N-Acetylpetroselinylamine

Many of these compounds had positive antimicrobial activity in this screening test. Mixed fatty acids from oil of Limnanthes Douglasii, in

contrast, showed no such activity.

*is important in this activity.*

#### Pharmacological Properties

Rat feeding tests on new crops and meals. Parsley seed oil, Limnanthes Douglasii seed oil and its fatty acids, petroselinic acid, and capric acid, as well as the solvent-extracted meal from parsley seed and Limnanthes Douglasii were submitted to the Pharmacology Laboratory, WU,





for rat feeding tests to determine calorie availability. The results of these tests as reported to us by Dr. A. N. Booth, are as follows:

In assay No. 7 it can readily be seen that both petroselinic and capric acid are very similar to corn oil as energy sources for the rat. The group weight gains for the 7-day period were +43, +36, and +41 grams respectively. Parsley seed oil, on the other hand, was not readily ingested by rats and was poorly utilized when compared to corn oil in assay No. 8 (+13 vs. +37). Perhaps deodorization of the parsley seed oil might make it more palatable for rats. Both Limnanthes seed oil and fatty acids were equally as good as corn oil (+46 vs. +35 vs. +37).

Regarding the solvent extracted meal from parsley seed and Limnanthes Douglasii, these have now been fed to rats (5 female weanlings per group) for a period of 85 days. Results are as follows:

Group	Average body weight		
	Start	49 days	85 days
1. Addis basal + 20% soybean meal	54.4	168	191
2. Addis basal + 20% parsley seed meal	52.4	163	187
3. Addis basal + 20% <u>Limnanthes</u> D. meal	52.4	144	173
4. Addis basal + 20% <u>Limnanthes</u> D. meal + 0.4% DL Methionine	52.8	143	discontinued

Addis basal = 73% corn meal, 10% crude casein, 10% linseed meal, 2% alfalfa meal, 3% cod-liver oil, 1.5% bone ash, and 0.5% salt.

From the above results it is concluded that parsley meal is as good as soybean meal based on growth. The slightly reduced growth of rats on 20% Limnanthes meal (173 vs. 191) was not improved by the addition of



Calorie Assay Number 7

Supplement (Gm/rat/day)	Wt. gain (Gm.) (5 rats/7 days)	Wt. change (gm.) on basal (5 rats/2 days)	Corrected net wt. gain* (Gm.) -	Moisture-free fecal wt.* (Gm.) -	Moisture-free urine solids* (Gm.) -
1. Basal only (5.0 gm.)	+15 (0)	+3 (0)	-	5.2	29.6
2. + D-glucose (0.5 gm.)	+38 (+23)	+1 (-2)	+21	5.2	27.7
3. + D-glucose (1.0 gm.)	+52 (+37)	-1 (-4)	+33	5.2	27.1
4. + Pectin (N.F.) (1.0 gm.)	+33 (+18)	-18 (-21)	- 3	Feces + urine wt. = 6.09 - 27.4 = 33.5 fecal wt.	
5. + Pectin (N.F.), (1.0 gm.) + neomycin (11 mg.)	+40 (+25)	-19 (-22)	+ 3	Feces + urine wt. = 64.1 - 27.4 = 36.7 fecal wt.	
6. + D-mannose (0.75 gm.)	+51 (+36)	- 5 (-8)	+28	7.2	30.5
7. + Wheat wafer (1.0 gm.)	+50 (+35)	- 2 (-5)	+30	7.3	29.6
8. + Sulfathalidine (15 mg.)	+22 (+7)	0 (-3)	+ 4	5.3	29.9
9. + Sulfathalidine (15 mg.) + neomycin (10 mg.)	+43 (+28)	+ 7 (+4)	+28	0.3	29.0
10. + Corn oil (0.5 gm.)	+56 (+41)	+ 3 (0)	+41	5.6	26.6
11. + Petroselinic Acid (0.5 gm.)	+59 (+44)	+ 2 (-1)	+43	5.9	27.3
12. + Capric Acid (0.5 gm.)	+61 (+46)	- 7 (-10)	+36	5.5	28.1

\* 5 male rats per group





Calorie Assay Number 8

Supplement (Gm/rat/day)	Wt. gain (Gm.) 5 rats/7 days	Wt. change (gm) on basal (5 rats/2 days)	Corrected net wt. gain* (Gm.) -	Moisture-free fecal wt.* (Gm.)	Moisture-free urine solids* (Gm.)
1. + Corn oil (0.5 gm.)	+44 (+38)	+4 (-1)	+37	7.0	28.1
2. + Cornstarch (1.0 gm.)	+40 (+34)	+3 (-2)	+32	7.0	28.0
3. + Parsley Seed Oil (0.5 gm.)	+20 (+14)	+4 (-1)	+13	5.1	(food spillage)
4. + <u>Limnanthes Douglasii</u> <u>Seed Oil</u> (0.53 gm.)	+52 (+46)	+5	+46	7.3	28.2
5. + <u>Limnanthes Douglasii</u> <u>fatty acids</u> (0.5 gm.)	+41 (+35)	+5	+35	8.3	28.9

\* 5 male rats per group.



methionine. In another experiment, not indicated above, Limnanthes meal was heated in an autoclave at atmospheric pressure for 30 minutes and fed to rats. Here again growth was slightly inferior to the control group, indicating no improvement due to heat treatment. Tissues including pancreas, thyroid and adrenals will be examined for evidence

In additional interpretation of these results, it may be noted that antimicrobial activity of petroselinic and capric acids is apparently not effective against intestinal microorganisms when they are ingested, since they are equivalent to corn oil but not superior.

Parsley seed oil was unpalatable to the rats and resulted in poorer weight gains than did corn oil. Parsley seed meal was comparable, but Limnanthes Douglasii meal somewhat inferior to soybean meal as food for rats. The crude fiber content of the parsley seed meal (15.6%) is almost twice that of the Limnanthes meal (8.0%) so this factor does not account for the poor showing of the latter. However, Limnanthes meal has been shown to contain a glucosidic material (Ettlinger, M. G., and Lundeen, A. J., J. Am. Chem. Soc. 70, 1952 (1956) "The Mustard Oil of Limnanthes Douglasii Seed, m-Methoxybenzyl Isothiocyanate.") which could affect its nutritional value.





### Future Research

Research will be continued on the development of methods for separating the individual fatty acids of Limnanthes Douglasii seed oil. Fractional distillation and preparative GLC, possibly in combination with crystallization or urea complexing, will be investigated further as means of purifying the more important fatty acids.

The chemical modification of the lactone which can be prepared from distilled eicosenoic acid will be investigated, since bond position and configuration are apparently unimportant in the lactonization reaction. When supplies of pure acids become available, one or more of the lines of anticipated work described in the 1961 report may also be initiated.

Further research will be carried out on chemical derivatives of capric acid, the major component of oil from seed of various Cuphea. During preparation of 2-decenoic acid by debromination of 2-bromodecanoic acid, not only was a mixture of 2-decenoic and 2-hydroxydecanoic acid obtained, but also an unknown third compound, as shown by GLC, which distills with the 2-decenoic acid. The identity of the third compound will be determined because of interest in the compound per se and because knowing the nature of the unknown may enable us to devise methods of obtaining higher yields of 2-decenoic and/or 2-hydroxydecanoic acid. This and other chemical derivatives of capric acid will be screened for antimicrobial activity and other biological properties.

Research on petroselinic acid and its derivatives will be continued, with emphasis on cooperation with the Crops Research Division to evaluate the potential of fennel seed and oil, and on ozonization and preparation of various chemical derivatives.



In order to evaluate fennel seed as a source of petroselinic acid they must be analyzed for oil and the petroselinic acid content of the oil determined. Methods used for the latter thus far require a correction factor because the conversions are not quantitative. An analytical procedure for ozonizing and reducing the ozonides of unsaturated fatty acids giving quantitative yields of the aldehydes has been recently reported by Privett and Nickell (J. Am. Oil Chemists' Soc. 39, 414 (1962)). The aldehydes were then determined by gas-liquid chromatography. The applicability of this method will be determined on samples of mixed fatty acids from fennel seed oils that have already been analyzed by ozonization, oxidation and chromatographic separation of adipic acid. Another approach which will be explored is the deuteration of petroselinic acid with deuterohydrazine and determination of its mass spectra.

The preparation of trimethylol undecane and 5,5,5-trimethylol valeric acid (or its lactone) from ozonized and oxidized petroselinic acid will be studied. The first compound from dodecanal has been prepared and neither of these compounds seems to have been reported in the literature.

#### Summary

Limnanthes Douglasii. Methods attempted for separation of the component fatty acids of oils of Limnanthes Douglasii have not been too successful thus far. Fractional distillation of the methyl esters resulted in a good yield of methyl eicosenoate but the yield of C<sub>22</sub> ester was poor and all fractions contained some trans bonds indicating isomerization during distillation. Further studies are being made to determine whether conditions can be established for separating the esters by distillation without their deterioration.

ethylene imide —  $\begin{array}{c} \text{O} \\ \parallel \\ \text{C} - \text{N} \begin{array}{l} \nearrow \text{CH}_2 \\ \searrow \text{CH}_2 \end{array} \end{array}$

would be reactive w cotton, starch, etc  
amides of hydroxy alcohols



Gamma-lactones have been prepared from Limnanthes Douglasii fatty acids in yields of about 80% theoretical with 70% perchloric acid as a catalyst, but only 65% conversion with p-toluenesulfonic acid as a catalyst. The latter, however, offers considerable advantage in safety. Limnanthes oil and fatty acids were equivalent to corn oil as energy sources for rats but the meal supported growth poorly.

Cuphea. Oils from Cuphea, having a high content of triglycerides containing 8 and 10 carbon fatty acids, could have pharmaceutical application in the treatment of patients having chyluria.

A literature survey revealed that numerous derivatives of capric acid showed interesting surface active properties and physiological activity. A number of compounds derived from capric acid have been screened for antimicrobial activity and found to be effective inhibitors of growth of a number of fungi.

Capric acid was investigated as a modifier for starch and although it was not particularly effective in modifying starch for conventional adhesive formulations, it appears that a highly esterified starch might find utility as a solvent soluble, dip or spray type of adhesive. Capric acid was found to be equivalent to corn oil as an energy source for rats.

Petroselinic Acid. The literature on petroselinic acid and its chemistry has been carefully reviewed.

Based on analysis of fennel seed submitted by Dr. J. R. Haun, New Crops Research Branch, Beltsville, Maryland, representing three different planting dates and three different spacings, the prospects of this plant as an oilseed crop would appear to be encouraging. Oil content of the seed ranged from 15.6 to 19% for the two earlier plantings and petroselinic acid content from 53.6 to <sup>62.1</sup>~~59.7~~% of the oil, *unreproducible range!*

*from 11 to 15%*



Trimethylol derivatives of aldehydes derived from new crops oils are interesting new compounds which should have potential for industrial use. Antimicrobial activity was observed for a number of organic compounds which can be readily derived from petroselinic acid. This is an interesting finding since it appears to be related to the position of the double bond and the fact that 6 and 12 carbon fragments would be formed on degradation. Apparently fatty derivatives of 6 and 12 carbon chain length have specific antibiotic activity not shared by related compounds. Mixed fatty acid of Limnanthes, for example, having the double bond primarily in the 5-6 rather than the 6-7 position, did not exhibit antibiotic activity when tested against the same organisms. Parsley seed oil was not sufficiently palatable to give good weight gains when fed to rats. Petroselinic acid and Limnanthes Douglasii fatty acids were equally as good as corn oil as energy sources. Antibiotic activity of petroselinic acid, however, did not appear to carry over when it is ingested. Parsley seed meal was as good a source of growth as soybean meal but Limnanthes meal was inferior and research may be needed to improve its nutritional quality should prospects for a crop from this plant materialize.





## Publications and Presentations

1. Sara P. Fore, T. L. Ward, and F. G. Dollear. "The Preparation of Lauryl Alcohol and Hydroxycaproic Acid from Petroselinic Acid," J. Am. Oil Chemists' Soc. (in Press).
2. R. L. Holmes, J. P. Moreau, and R. T. O'Connor. "Preparation of Dodecylamine and 6-Aminohexanoic Acid from Petroselinic Acid," J. Am. Oil Chemists' Soc., 39, 411-414 (September 1962).
3. Lida L. Placek and F. G. Dollear. "The Preparation and Properties of Some Nitrogen-Containing Derivatives of Petroselinic Acid," J. Am. Oil Chemists' Soc., 39, 347-350 (August 1962).
4. Lida L. Placek. "A Review on Petroselinic Acid and Its Derivatives," Presented at the 142nd National Meeting of the American Chemical Society, September 9-14, 1962.

## Patent Application

1. Robert R. Mod, Evald L. Skau, Sara P. Fore, Frank C. Magne, Arthur F. Novak, Harold P. Dupuy, Jesse R. Ortego, and Mary J. Fisher. "Certain Derivatives of Aliphatic Acid Amides." Filed May 24, 1962.

*Examined 197, 548 One division has been  
allowed (Jan, 1964)*



Western Utilization Research and Development Division  
 Division Report for Annual New Crops Meeting  
 Washington, D. C., January 25, 1963

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During this period it was recognized that the plant material previously identified as Dimorphotheca ecklonis is more properly designated as Osteospermum ecklonis. It is apparently very closely related to Dimorphotheca and the oil from the seed contains a high proportion, ca. 65%, of dimorphecolate. This report concerns our work on Dimorphotheca, and Osteospermum (source of 9-hydroxy-10, 12-octadecadienoic acid) and on Lesquerella (source of 14-hydroxy-11-eicosenoic acid).

Processing of seed. Processing of about 200 pounds of seed of Dimorphotheca by milling, air classification to remove hulls, grinding and solvent extraction with Skellysolve F has been described previously. In the case of Lesquerella the following was found to be a more convenient procedure: The seed was cleaned by passing through a Simon-Carter indent separator (more than 80% recovery of quite clean seed), then cracked (not crushed) by passing between rollers. (Rolls of an available rubber mill were used and set for a clearance of 0.005 inch.) The cracked seed (5 kg.) was then stirred with 5 liters of naphtha (Skellysolve B) at 65°C. for 1 hour and filtered through a Buchner funnel. The residue was re-extracted for 15 minutes with additional naphtha and again filtered. The combined filtrates gave a good quality oil (no fines and less than 2% free fatty acid as oleic acid) in a yield of better than 24% based on the weight of dry, cleaned seed used. Analysis by infrared absorption at 2.76 microns indicated the presence of about 60% lesquerolate in the oil. This appears to be a very satisfactory, rapid, large-scale laboratory extraction procedure. No unusual problems are anticipated in converting this to larger scale processing, e.g., by flaking and solvent extraction of the flakes with the usual hydrocarbon solvents. The recovery of residual meal was substantially quantitative (75%). Analysis indicated the presence of 5.45% nitrogen (34% protein) and about 1.25% crude fat, 12% crude fiber and 9% ash.

Analysis of seed. Analysis of the "44 samples of four known and two unspecified samples" of Dimorphotheca seed obtained by Crops Research from experimental plots referred to in last year's report was completed. However, it appears that these samples consisted of only two species, D. pluvialis and D. sinuata. The species previously identified as D. annua is in reality D. pluvialis and the species previously designated as D. aurantiaca, calendulacea and sinuata are all properly designated D. sinuata. For 9 samples of D. pluvialis the mean value for recovery of dehulled seed was 38.6%, for percent oil on whole seed was 10.9 and on dehulled seed was 28.0; the percent dimorphecolic acid derivable from the oil was 40.5 and the percent crude protein in the meal from the dehulled seed was 50.3. For 29 samples of D. sinuata the mean value for recovery of dehulled seed was 37.1, for percent oil on whole seed was 11.2 and on dehulled seed was 29.5; the percent dimorphecolic acid derivable from the oil was 48.2 and the percent crude protein in the meal from the dehulled seed was 55.4. The highest values were obtained from D. sinuata



seed grown at Corvallis, Oregon. These were: 46.6% dehulled seed, 15.0% oil on whole seed and 33.9% on dehulled seed, 59.6% dimorphecolic acid derivable from the oil and 59.7 crude protein. A draft of a manuscript reporting this work has been prepared in cooperation with Crops Research.

Five additional samples of Dimorphotheca seed received from the Chico Station were similarly analyzed. The results were comparable to those obtained with earlier samples except that one sample afforded 49.6% dehulled seed, 19.24% oil on whole seed and 38.5% oil on dehulled seed and 63.8% derivable dimorphecolic acid from the oil. These are the highest values obtained on any sample of Dimorphotheca seed yet analyzed.

Four samples of Osteospermum ecklonis seed, supplied by the Chico Station were analyzed in a similar manner. The proportion of dehulled seed ranged from 38.8 to 48.7%; the percent oil in the whole seed ranged from 17.4 to 22.8% and the percent derivable dimorphecolic acid (as judged by conjugated diene content) ranged from 59.6 to 69.9%. This species, therefore, seems superior to Dimorphotheca as a source of dimorphecolic acid as even the lowest of the values for oil and dimorphecolic acid content are equal to or higher than the highest values for any of the more than forty samples of Dimorphotheca seed analyzed. Also, the seed is distinctly larger and the proportion of hull is generally smaller.

Several samples of cleaned seed of Lesquerella fendleri were analyzed and the following average values (dry basis) were obtained: Percent oil in seed, 25.8; hydroxy acid content (as lesquerolic acid) 60%; percent meal, 74.1. The meals contained an average of 5.45% nitrogen (34.1% crude protein), 1.25% crude fat, 12.0% crude fiber and 9.2% ash.

Several samples of oilseed meals were analyzed for amino acid content using the Moore-Stein technique (Beckman-Spinco analyzer). The results are shown in Table I. The checks between NU and WU are fairly good in most cases. However, serious discrepancies in cystine content and the fact that in all cases low recoveries were obtained of total nitrogen as amino acids or ammonia point to the need for research on methods of hydrolysis specifically adapted to the meals in question.





Table I

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Amino acid analyses on meals from hydroxy-type oilseeds.

	<u>D. aurantiaca</u> Sample 5021 Mean of 4 runs	WU-NU check samples			
		<u>L. fendleri</u>		<u>O. ecklonis</u>	
		NU	WU	NU	WU
lysine	3.66	6.6	6.0	3.4	3.5
histidine	1.82	2.5	2.4	2.0	2.0
ammonia	2.02		2.0		2.2
arginine	6.38	7.9	7.2	7.6	8.0
aspartic acid	7.98	7.2	7.1	8.9	9.3
threonine	2.99	4.4	4.4	3.0	3.1
serine	3.36	4.6	5.6	3.5	3.5
glutamic acid	15.20	13.7	13.9	16.5	17.5
proline	3.86	6.7	6.3	3.5	3.6
glycine	4.99	5.9	6.2	4.6	5.0
alanine	3.39	4.5	4.7	3.5	3.6
cystine	1.64	1.2	0.9	1.7	3.2
valine	3.87	4.8	4.8	4.4	4.5
methionine	1.20	1.4	1.3	1.1	1.2
isoleucine	3.55	3.6	3.5	4.0	4.0
leucine	5.47	5.8	6.3	5.8	6.0
tyrosine	2.43	3.0	3.0	2.5	2.5
phenyl alanine	3.14	3.8	3.9	3.4	3.7
% proteins (N x 6.25)	55.6	28.3	28.3		52.8
Recovery of N	79.0	77.4	77.9	71.6	70.9

low recoveries

W or Ethen uses separate procedure for cystine if he really wants accurate value.

Hydrolyses - need more methodology work - Solidstate.

W & E - Sulfuric acid hydrolysis under pressure - might inhibit browning reaction.



Composition of Dimorphothecca oil. A detailed examination of the composition of the Dimorphothecca oil from a very good sample of seed of D. sinuata (aurantiaca) was made. The non-saponifiable content of the oil averaged 1.3%. The fatty acid composition was determined with the aid of partition chromatography on silicic acid wetted with methanol-benzene, GLC, TLC, and ultraviolet, infrared and NMR spectroscopy. Methyl esters used for analysis, e.g., by GLC, were prepared both by esterification of the free fatty acids with diazomethane and by anion exchange methanolysis of the Dimorphothecca oil. The diazomethane reaction appears to introduce some artifacts, e.g., formation of the methyl ether of the secondary hydroxyl (9-methoxy) comparable to the formation of 12-methoxy oleate previously observed when castor acids are esterified with diazomethane. Methanolysis does not remove the non-saponifiables (1.3%) which subsequently causes certain difficulties in the GLC analysis. In general, the composition previously reported by NU and by L. J. Morris, et al., (Hormel Institute) was confirmed but with some significant differences. Specifically, for example, about 2-1/2% of the previously unreported 9-keto-10,12-octadecadienoic acid was found. That this acid is present in the original oil and is not an artifact was established by noting the characteristic bathochromic shift in absorption at about 270 millimicrons when the solvent for the Dimorphothecca oil, as well as the isolated methyl ester of the ketodiene, was changed from cyclohexane to methanol. The presence of about 1/2 percent of epoxides in the methyl esters, previously reported by Morris, was confirmed and firmly established by NMR (Dr. Lundin, Wool Lab) but only two epoxy acids could be recognized by TLC instead of the five reported by Morris. The total conjugated diene content of the Dimorphothecca acids corresponds very closely to the total hydroxyl content, from which it may be inferred that all of the hydroxyl and of the conjugated diene is present as dimorphecolic acid and this is supported by detailed examination of various fractions obtained by partition chromatography with silicic acid. Our best estimates for the fatty acid composition of this Dimorphothecca oil are given in Table II.





Table II

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% Fatty acids in mixed Dimorphotheca sinuata

Acid	Weight, %
Dimorphecolic	65.5
Linoleic	14.3
Oleic	10.0
Palmitic	2.9
9-Keto-10,12-octadecadienoic* ( <u>tt</u> )	2.4
Stearic	1.7
Octadecadienoic <sup>tr</sup> (Conj.)	1.3
Linolenic	0.5
Minor components, (0.1 to 0.5%)**	1.2
Minor components, (< 0.1%***	<u>0.2</u> 100.0

\*Identified by comparison with authentic 9-keto diene prepared by oxidation of dimorphecolic acid and the derived 9-ketostearic acid.

\*\*Identified chiefly through ECL on polyester and apiezon GLC columns: myristic (0.3); arachidic (0.3); behenic (0.2); epoxy C18:1 (0.3) and C18:2 (0.1).

\*\*\*Palmitoleic (0.05); nonadecanoic (0.05); heptadecanoic (0.03) [ECL 17.7 (0.03)]; pentadecanoic (0.02).



Analytical constants for dimorphecolic and lesquerolic acid. In order to be able readily to determine quantitatively the dimorphecolate and lesquerolate content of various oils and reaction products it was necessary to determine various physical constants, especially ultraviolet and near infrared absorption coefficients for the pure compounds. Accordingly, small samples were purified by various techniques, including crystallization, distillation, liquid-liquid extraction and partition chromatography. Of particular utility for analysis are the following molar absorptivities: methyl dimorphecolate --  $\epsilon = 33,700$  at  $231 \text{ m}\mu$  (for diene content);  $\epsilon = 55.2$  at  $2.76 \mu$  (for hydroxyl content); methyl 9-hydroxystearate --  $\epsilon = 41.2$  at  $2.76 \mu$ ; methyl 9-keto-10, 12-octadecadienoate --  $\epsilon = 28,600$  at  $268 \text{ m}\mu$  in cyclohexane and  $29,100$  at  $275 \text{ m}\mu$  in methanol; methyl lesquerolate --  $\epsilon = 28.5$  at  $2.76 \mu$  and for methyl 14-hydroxyeicosanoate  $\epsilon = 39.9$  at  $2.76 \mu$ .

A series of tests to determine whether the characteristic absorption at  $2.76$  microns can be used for determination of hydroxyl content indicated that for these compounds Beer's Law is obeyed at concentrations below about  $0.4\%$ . At this concentration the observed optical density is in the range  $0.4$  to  $0.8$  for the pure compounds when measured in  $1$  or  $2 \text{ cm.}$  cells. This characteristic absorption can, therefore, be used to determine the hydroxyl content of these materials. Since the molar absorptivities of the hydroxy acids differ the hydroxyl content of mixtures of these hydroxy acids cannot be determined solely from the characteristic absorption at  $2.76$  microns, i.e., the hydroxyl content of a mixture of lesquerolic and hydroxyeicosanoic acid cannot be determined in this way. Fortunately, however, the presence of more than one hydroxy acid has not been observed in any of the Dimorphotheca or Lesquerella oils we have used.

To aid in the identification of mixtures containing conjugated diene, triene and keto-diene, appropriate synthetic mixtures of methyl dimorphecolate, methyl alpha eleostearate and methyl 9-keto-10,12-octadecadienoate were made up and their ultraviolet absorption spectra were examined. The following observations are believed significant: At a concentration of  $20\%$  conjugated diene (remainder triene) a barely detectable maximum can be observed at  $234 \text{ m}\mu$  and the three maxima at about  $260$ ,  $270$  and  $280 \text{ m}\mu$  characteristic of conjugated triene are well defined; at  $30\%$  diene the normal maximum at  $231 \text{ m}\mu$  is clearly apparent as is also a "false maximum" at  $238 \text{ m}\mu$ . This "false maximum" is no longer apparent at a concentration of  $40\%$  diene. The three maxima characteristic of triene are fully apparent until the diene content is raised to  $90\%$ , but above this the maximum at  $258 \text{ m}\mu$  almost disappears. In mixtures of triene and keto-diene -- the spectrum in methanol appears practically identical with that of pure triene if only  $10\%$  keto-diene is present; at a level of  $30\%$  the peak at  $258 \text{ m}\mu$  appears as a barely discernible maximum at  $260 \text{ m}\mu$ ; at  $90\%$  keto-diene a broad peak is obtained with barely observable maxima at  $271$  and  $278 \text{ m}\mu$  and these disappear completely at the  $95\%$  level of keto-diene.





Concentration of dimorphecolic and lesquerolic acid. In order to be able better to analyze chemical reaction products and to purify, characterize and evaluate them, it is desirable to start with the acids (or esters) of greater concentration (purity) than that of the whole mixed acids of the oils themselves, ca. 60%. Although partition chromatography on silicic acid is an excellent method for preparing very pure samples of dimorphecolic and lesquerolic acid (and their methyl esters) this method is too cumbersome to use in preparing the larger quantities desired for routine laboratory use. A more convenient way to concentrate dimorphecolic acid (as the methyl ester) was found to be by partition between suitable pairs of immiscible solvents. Suitable pairs of solvents include Skellysolve (F or B) and either acetonitrile or aqueous methanol. Partition of mixed methyl esters of Dimorphotheca oil between Skellysolve F and acetonitrile (5 ml. of each solvent per gram of mixed esters) in a simple, manual, 3-stage extraction procedure afforded more than 90% of the methyl dimorphecolate present in greater than 90% purity. Similar results were obtained by partitioning the mixed esters between Skellysolve F and 92% aqueous methanol.

Attempted partitioning of mixed methyl esters of Lesquerella oil was much less successful, presumably owing to the greater chain length and, hence, increased solubility of methyl lesquerolate in the hydrocarbon solvent. Thus partitioning of the mixed methyl esters of Lesquerella oil between Skellysolve F and acetonitrile afforded a recovery of only about 30% of the methyl lesquerolate with a purity greater than 90%. Comparable results were obtained using Skellysolve F and 92% aqueous methanol. Fortunately, however, the methyl lesquerolate can conveniently be concentrated by fractional distillation at reduced pressure. (This is not feasible for purifying methyl dimorphecolate because it dehydrates excessively at the temperature required for distillation.) Methyl lesquerolate distills at about 190° at 50 microns while the non-hydroxylated components of the mixed esters distill below about 150° at this pressure. Good recovery of methyl lesquerolate of better than 95% purity was obtained by such distillation. However, care must be taken to avoid excessive pressures (and, hence, distillation temperatures) as methyl lesquerolate dehydrates appreciably to diene at pot temperature above about 230°.

→ Dehydration of Dimorphotheca and Lesquerella oil. The catalyzed dehydration of Dimorphotheca and Lesquerella oil was investigated. Using castor oil as a reference oil it was found that best results, i.e., based on loss of hydroxyl, proportion of conjugated diene, good color, and low viscosity, were obtained using 1% by weight of NaHSO<sub>4</sub> as a catalyst and a temperature of 250-255°. Under these conditions, most of the dehydration occurred in 15 minutes but generally the dehydration was continued for an additional 3/4 hour with samples taken at 15-minute intervals. Typically, after an hour, less than 10% of the original hydroxyl content remained (as judged by characteristic absorption at 2.76 microns) and the product, which was pale yellow in color, contained about 30% conjugated diene (as conjugated linoleic acid) and had a viscosity of G-H on the Gardner scale (1.7 poises as against U-V or 6.9 poises for the original oil). Lesquerella oil, when similarly treated with 1% NaHSO<sub>4</sub>, dehydrated in an exactly analogous manner. The largest proportion of the dehydration occurred in the first 15 minutes but the reaction was continued for an additional 3/4 hour further to



decrease the hydroxyl content. At that time the hydroxyl content was less than 10% of that originally present and the viscosity was E-F (1.4 poises as against I-J or 2.4 poises for the original oil). The conjugated diene content of the oil compared well with that of the dehydrated castor oil after making allowance for the smaller proportion of hydroxy acid in the Lesquerella oil and the smaller extinction coefficient to be expected from a C20 as compared to a C13 conjugated diene.

Dimorphotheca oil could be dehydrated much more readily than either Lesquerella or castor oil. Thus after heating with 1%  $\text{NaHSO}_4$  for 45 minutes at a temperature of only 110-120° less than 10% of the original hydroxyl content remained. Ultra-violet absorption spectra indicated the presence of about 65% conjugated triene, as eleostearic acid, i.e., substantially quantitative conversion to conjugated triene. The viscosity was K-L (2.9 poises as compared to U-V or 7.5 poises for the original oil) and the color was a good, very pale yellow.

These dehydration products appear to be distinctly better than those reported by Rheineck (Official Digest, Fed. Soc. Paint Tech. 34, 862-883 (1962)) who reported that refluxing with glacial acetic acid afforded a product with J viscosity which contained some residual hydroxyl, 21.1% conjugated diene and 29.2% conjugated triene, and that treatment with 2%  $\text{KHSO}_4$  at 115° gave a product which contained some hydroxyl, 25.6% conjugated diene and 9.2% conjugated triene. Nevertheless, in Professor Rheineck's report of his Study of the Film Forming Characteristics of Dimorphotheca oil he concluded that "Comparable phenolic resin varnishes of Dimorphotheca and tung oil showed only marginal differences in their performance characteristics." This conclusion speaks well for the potential of Dimorphotheca oil as a drying oil.

Pyrolysis of methyl lesquerolate. The pyrolysis of methyl ricinoleate to produce heptaldehyde and methyl undecylenate is a process used commercially on a relatively large scale. The pyrolysis of methyl lesquerolate was investigated briefly. Using methyl ricinoleate as a model reference material substantial yields of heptaldehyde and methyl undecylenate were obtained by passing the methyl ricinoleate through a layer of molten lead alloy at a temperature of 550-560°. Similar treatment of methyl lesquerolate afforded heptaldehyde and methyl tridecylenate, identified through various physical characteristics and by GLC. Maximum yields obtainable from methyl lesquerolate have not yet been determined but in the case of methyl ricinoleate the yield of methyl undecylenate, based on the proportion of methyl ricinoleate decomposed, was nearly theoretical. Optimum conditions for conversion of methyl lesquerolate are now being determined.

Phosphorus containing derivatives. Considerable effort was spent on the preparation of various phosphorus containing derivatives of potential utility in flame retardants, surface coatings, plasticizers and hydraulic fluids. Use of various phosphorylating agents was investigated, including phosphorus trichloride ( $\text{PCl}_3$ ), phosphorus oxychloride ( $\text{POCl}_3$ ), dialkyl phosphorochloridates ( $(\text{C}_2\text{H}_5\text{O})_2\text{P}(\text{O})\text{Cl}$  and  $(\text{C}_4\text{H}_9\text{O})_2\text{P}(\text{O})\text{Cl}$ ), and dimethyl phosphites ( $(\text{CH}_3\text{O})_2\text{PH}(\text{O})$ ). Methyl ricinoleate was used as a model reference compound and certain phosphorus containing derivatives





were prepared from the methyl esters of ricinoleic, dimorphecolic, lesquerolic, 9-hydroxystearic, 12-hydroxystearic and 14-hydroxyeicosanoic acid. In the course of this work, formation of the lactones of ricinoleic and 12-hydroxystearic acid, under certain conditions, was noted and these lactones were isolated and characterized.

One of the oldest methods for preparing phosphorus esters is by reaction between phosphorus halide and a sodium alcoholate, the salt being prepared by treating the alcohol with metallic sodium. This latter reaction is unsuitable when applied to esters of hydroxy fatty acids, since the undesired Claisen ester condensation would occur simultaneously. Alternatively, an organic base may be used as an HCl-acceptor in the reaction between the alcohol and phosphorus halide. Reaction of methyl ricinoleate with  $\text{PCl}_3$  in an inert solvent with N,N-diethylaniline ( $48^\circ\text{C}$ ., 1-1/2 hours) afforded a phosphorus containing product estimated to consist of about 75% triester,  $(\text{RO})_3\text{P}$ , and 25% diester,  $(\text{RO})_2\text{PH}(\text{O})$ , (R is  $\text{C}_{19}\text{H}_{35}\text{O}_2$ ). Analogous reaction of methyl ricinoleate with  $\text{POCl}_3$  even under more severe conditions ( $71^\circ\text{C}$ ., 2 hours) afforded only a small yield of the triester. When methyl dimorphecolate was treated in a similar manner with either  $\text{PCl}_3$  or  $\text{POCl}_3$  dehydration occurred, conjugated triene was obtained and no long chain phosphorus containing esters could be isolated.

Many mixed esters are commonly prepared by reaction of a hydroxylic compound and a dialkyl phosphorochloridate,  $(\text{RO})_2\text{P}(\text{O})\text{Cl}$ , in the presence of a basic HCl-acceptor. However, when methyl ricinoleate was treated with diethyl- or dibutyl phosphorochloridate under a wide range of commonly used reaction conditions infrared analysis indicated no decrease in hydroxyl content and, hence, no desired reaction. Reaction conditions varied included temperature ( $25$  to  $113^\circ\text{C}$ .), time (1 to 94 hours), base ( $\text{K}_2\text{CO}_3$ , triethyl amine, 2,6-lutidine) and solvent (hydrocarbon, ether, ketone, acetonitrile). However, when methyl ricinoleate was treated with diethyl phosphorochloridate and triethyl amine according to a recently published procedure (Ber. 94, 989 (1961)) with acetonitrile as a solvent and dimethylformamide as catalyst, conversion to the mixed phosphate triester,  $(\text{C}_2\text{H}_5\text{O})_2\text{P}(\text{O})\text{OR}$ , was obtained even at room temperature. Attempted purification of the product (about 75% triester) by crystallization followed by chromatography on silicic acid was only partially successful. Analogous reaction of diethyl phosphorochloridate and methyl dimorphecolate at room temperature or slightly below resulted in the formation of considerable proportions of the corresponding triester, with little dehydration as evidenced by infrared and ultraviolet spectra, but extensive dehydration to conjugated triene occurred during attempts to purify the desired triester. Some of the desired triester was obtained fairly pure (ca. 90%) by partitioning the reaction product between acetonitrile and petroleum ether but this product decomposed spontaneously.

Another mode of preparation of phosphorus containing triesters, and the one found to be much the most satisfactory except for dimorphecolate, is the transesterification between dimethyl phosphite and the methyl ester of the hydroxy fatty acids. In the case of methyl dimorphecolate excessive dehydration occurs, as evidenced by formation of conjugated triene. Effects of mole ratio of reactants



and of temperature and time of reaction on the nature of the reaction products were investigated. The phosphate esters obtained were purified by partitioning between acetonitrile and commercial pentane and also by chromatography with silicic acid.

The transesterification reaction was carried out between dimethyl phosphite and each of the following methyl esters of long chain fatty acids: ricinoleic, dimorphecolic, lesquerolic, 9-hydroxystearic, 12-hydroxystearic, and 14-hydroxyeicosanoic. In each case (except for dimorphecolic) various proportions (depending on conditions used) of the long chain diesters,  $(RO)_2PH(O)$ , and of the mixed diesters,  $ROPH(O)OCH_3$ , were obtained. It was noted that prolonged reaction time, particularly at higher temperatures, resulted in a decrease in the proportion of more polar products (extracted by acetonitrile) and increase in less polar products (extracted by petroleum ether) presumably by formation of lactones and estolides. In fact, the lactones of 12-hydroxystearic acid (stearolactone) and ricinoleic acid were isolated as by-products of the reaction when the methyl esters of these acids were used as reactants.

All the purified phosphorus esters were liquids, except the mixed methyl phosphite diesters obtained from methyl 12-hydroxystearate and methyl 14-hydroxyeicosanoate. It is planned to have some of the phosphite diesters and other phosphorus esters obtained from the glyceride oils evaluated as vinyl plasticizers (EU, Baker Castor Oil Company) as an antioxidant (Victor Chemical Company) and in metal primers (Professor Rheineck, North Dakota State College).





Urethane foams. The preparation of strong, light weight, fluorinated hydrocarbon blown foams using as the polyol component a Dimorphotheca oil-triisopropanol amine mixture of equivalent weight 100 was reported last year. It was felt that the unique drying properties of Dimorphotheca oil might prove advantageous in providing increased ultimate strength. Tests on a set of foam samples, made shortly after preparation and after ageing up to 7 months indicated very little, if any, change for the first month but an increase of about 10% in compressive strength was noted after three months with no significant further increase after an additional four months (40.9 to 41.4 p.s.i. compressive strength).

Foams prepared using Lesquerella oil/triisopropanol amine mixture of equivalent weight 100 as the polyol component appeared very similar to those obtained from Dimorphotheca oil and both appeared to be comparable to analogous castor-based foams.

Biological activity of Dimorphotheca and Lesquerella meal and oil. In cooperation with the Pharmacology Laboratory (WU) the nutritive value of the meals remaining after extracting the oil from Dimorphotheca and Lesquerella seed was examined, using rats and mice as the test animals. In preliminary experiments with young male mice with raw and heated Lesquerella meal as the sole source of protein the mice survived but they lost weight. Weanling rats fed with 20% raw and heated Lesquerella lasiocarpa meal added to the basal diet gained weight but not as much as those that were fed a standard basal diet as a control. Dimorphotheca meal was evaluated as a potential feed by comparing the weight gain of rats ingesting the meal with the gain produced with soybean meal. The results with Dimorphotheca meal were decidedly inferior to those with soybean meal (smaller gain in weight) and there was no improvement with methionine (0.4% of d,l-) and lysine (0.2%) were added. Toasting the meal to eliminate heat-labile factors was also ineffective. The food intake of the rats fed Dimorphotheca meal was appreciably reduced but it is not known at this time whether factors other than palatability are involved.

In a chick experiment Dimorphotheca meal (D.O.M.) was progressively substituted for 25, 50, 75 and 100% of the soybean oil meal of the ration on an isonitrogenous basis. Based on the amino acid analyses shown in Table I, it was considered that lysine would quickly become limiting for growth. Hence, synthetic lysine was added to bring the D.O.M. up to soybean meal in lysine content. The results showed that 25% of the soybean meal protein could be replaced with lysine fortified D.O.M. At higher percentage substitutions, growth and feed efficiency were depressed drastically. This result is reminiscent of the results obtained using raw soybean meal and points to the need for further studies on amino acid availability and the possible presence of inhibitors.

Also in cooperation with the Pharmacology Laboratory, tests were made to determine possible cathartic activity of the oils. Lesquerella oil, when fed rats by stomach tube, is definitely active as a cathartic, but the threshold value for cathartic activity appears to be of the order of twice that for castor oil, even after making allowance for the smaller hydroxy acid content of Lesquerella oil



as compared to castor oil. It may be significant that the first 9 carbon moiety (counting from the methyl end) of lesquerolic acid is identical with that of ricinoleic acid of castor oil; the only difference is that in lesquerolic acid the chain between the carboxyl group and the double bond is two carbons longer than it is in ricinoleic acid. Dimorphotheca oil, when fed rats by stomach tube, was also found to act as a cathartic but was less effective than Lesquerella oil. Dimorphotheca oil when added to a basal diet did not have any cathartic effect (nor did castor oil). The rats thus fed gained weight but not as much as rats fed the basal diet with either corn oil or castor oil. The gain in weight of the rats fed the diet to which castor oil had been added was substantially the same as that of the rats which received corn oil.

In the course of this work, methyl lesquerolate was converted to the trans derivative by isomerization, acetylated to 14-acetoxy-cis-11 eicosenoate and hydrogenated to 14-hydroxy-eicosanoate and the compounds characterized but their cathartic activity (or lack thereof) has not been fully established.

Contract research. Under a contract with the University of Arizona, Tucson, Arizona (Dr. C. S. Marvel) polymerization of various polymerizable monomers derived from long chain hydroxy fatty acids is being investigated. In particular, vinyl 12-hydroxystearate (derived from castor oil) polymerized by itself or copolymerized with co-monomers such as vinyl chloride or vinyl acetate afforded polymers ranging from waxy or hard solids to rubbery copolymers. Vinyl 9-hydroxystearate derived from Dimorphotheca oil was synthesized and has been submitted to the contractor for polymerization studies. Synthesis of vinyl 14-hydroxy-eicosanoate, from Lesquerella oil, is under way. Synthesis of other suitable monomers derivable from Dimorphotheca and Lesquerella oil is planned.

A contract with the Coatings Technology Department of North Dakota State College, Fargo, North Dakota, (Prof. A. E. Rheineck) has been negotiated. Under this contract surface coatings derivable from Dimorphotheca and Lesquerella oil will be prepared and evaluated.

Seed procurement. To supply our needs for the next twelve months the New Crops Research Branch (C. O. Erlanson) has been requested to supply 400 pounds Dimorphotheca seed of any available species, 100 pounds of Osteospermum ecklonis and 200 pounds of Lesquerella seed.





## SEED MEAL SCREENING FOR AMINO ACID COMPOSITION

This program was continued by determining the amino acid composition of seed meals from 74 species from 31 families. Seed from 15 species were high in lysine (5.3 to 7.2 grams per 16 grams N). The legumes Cicer arietinum contained 7.2 g. per 16 g. N; Cajanus cajan 6.8 and Lens culinaris 6.6. Seed from 14 species were high in methionine (2.1 to 2.6 g. per 16 g. N). Seed from Cephalotaxus harringtonia (CPR Poor) high in both lysine and methionine, contained 6.3 and 2.3 g. per 16 g. N, respectively. Seed from two species of the Proteaceae family contained high arginine (19 to 20 g. per 16 g. N). Seed from Peganum harmala (CPR Fair) contained an unknown imino acid, not detected in previous seed meals, present as 1.5 g. per 16 g. of nitrogen calculated as proline. Seed from Stizolobium deeringianum (CPR Fair) also contained an unknown not previously observed which was present as 6.2 g. per 16 g. of N calculated as leucine. Seed from these plants and from previously analyzed Staphylea pinnata (CPR Poor) (two unknowns, 10 g. per 16 g. N as leucine and the second as a shoulder on aspartic acid); Diospyros virginiana (CPR Poor) 1.4 g. N as leucine); Hemerocallis fulva (CPR Fair) (9.8 g. per 16 g. N as leucine), and Millettia ovalifolia (CPR Poor) (3.6 g. per 16 g. N calculated as proline) contain unidentified compounds the ninhydrin color yield of which indicate they are present in large amounts. To this date a total of seed meals from 349 species have been analyzed for amino acid composition.

The amino acid composition of two seed meals of special interest is provided in detail below:

Hexane-extracted seed meal from Onobrychis aurantiaca containing 60.5 percent (as-is basis) crude protein had the following amino acid composition expressed as g. per 16 g. nitrogen.

Lysine	4.9	Threonine	3.0
Methionine	1.7	Valine	3.7
Arginine	10.6	Alanine	3.3
Glycine	5.0	Aspartic acid	10.7
Histidine	4.3?	Glutamic acid	14.2
Isoleucine	3.3	Hydroxyproline	0.0
Leucine	5.4	Proline	3.1
Phenylalanine	3.0	Serine	4.0
Tyrosine	2.8		

Amino acid composition of hexane-extracted seed meal from Crepis foetida (28.7 percent crude protein, as-is basis) was as follows expressed as g. per 16 g. nitrogen:

Lysine	5.3	Threonine	4.1
Methionine	2.6	Valine	5.3
Arginine	7.0	Alanine	4.4
Glycine	6.0	Aspartic acid	8.3
Histidine	2.2	Glutamic acid	19.0
Isoleucine	4.0	Hydroxyproline	0 <del>6</del> .5
Leucine	6.5	Proline	5.0
Phenylalanine	4.8	Serine	4.2
Tyrosine	2.7		

Comparative results of amino acid analyses on check samples. The same samples of hexane-extracted seed meals from Osteospermum ecklonis, O. spinescens and Lesquerella fendleri were analyzed at WU and NU on the Beckman Spinco automatic analyzer. At NU the samples were acid hydrolyzed (0.5 g. in 250 ml. of 6 N hydrochloric acid) under reflux for 24 hours. At WU the samples were acid hydrolyzed (4.0 mg. in 10 ml. of 6 N hydrochloric acid) in a sealed tube in an oven at 110° C. for 22 hours. Analytical values were in excellent agreement between the two laboratories except for the following: In O. ecklonis, cystine NU 1.7, WU 3.2; glutamic acid NU 16.5, WU 17.5; in O. spinescens, arginine NU 8.0; WU 7.3; in L. fendleri, lysine NU 6.6, WU 6.0; arginine, NU 7.9, WU 7.2; serine, NU 4.6, WU 5.6. These values were not in marked disagreement except for cystine. It is known that acid hydrolysis destroys part of the cystine.

Testing of canavanine and a seed extract containing canavanine for antimicrobial activity. Canavanine is an antimetabolite of arginine, an amino acid which is essential for the growth of some microorganisms. For this reason the Fermentation Laboratory of NU at our request assayed a water extract of Sesbania macrocarpa seed containing canavanine and crystalline canavanine isolated from S. exaltata seed for antimicrobial activity against 21 selected test organisms. Canavanine showed no antibacterial activity. It has some antifungal action in chemically defined but not in nonchemically defined media.

Under 3-hydroxyproline up to ca. 3% in some materials.

## NEW SEED POLYSACCHARIDES

I. Viscosity Characteristics of *Cassia marilandica* gum

A limited study was made of the viscosity characteristics of the dry-milled gum from *C. marilandica* (CPR Good) (reported last year). This product forms stable, viscous solutions when dispersed in water (Figures 1 and 2). Viscosity of a 1-percent *C. marilandica* gum solution is approximately 3,000 centipoise; a similarly prepared guar gum solution has a greater viscosity of 6,800 centipoise.

II. Screening for New Seed Sources of Gum

Ninety new species of seed have been surveyed for gum content. These were picked primarily from the legumes because previous experience indicated this to be the most promising family. Those containing more than 18 percent water-soluble gum are shown in Table I. The most promising leads for agronomic followup are *Sphinctospermum constrictum*, *Cassia uniflora*, *C. biflora*, *C. leptocarpa*, and *C. leptadenia*. Certain others which appear promising in the table are discussed below. Toxic seeds are not being considered for further evaluation.

III. Toxicity Studies on Gum-Bearing Seeds

Fractions of *Cassia marilandica* seed were fed 7 days at 20 percent of the rat diet (Table 2). The gum is innocuous, but both hull and germ are toxic at this high level.

J. Wolff reporting on work of Dr. Turkey.

Crotolaria last yr decided to begin to pursue. 90 new species screened for gum content. Suggest feeding tests on Cassia. To avoid getting into same position as w. Crotolaria, *C. marilandica* viscosity somewhat lower than Crotolaria.





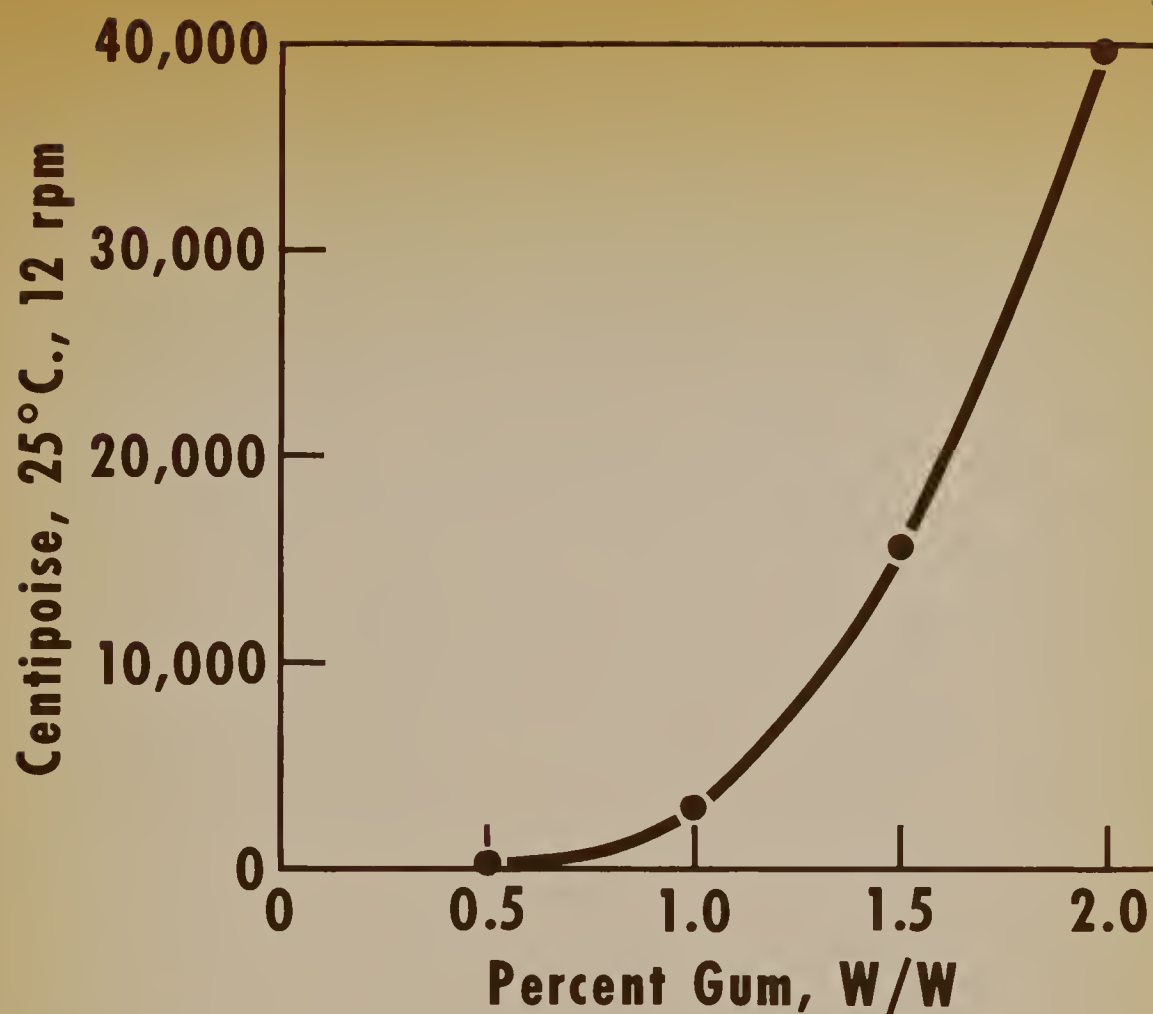


Fig. 1. Viscosity vs. Concentration of *Cassia marilandica* Gum

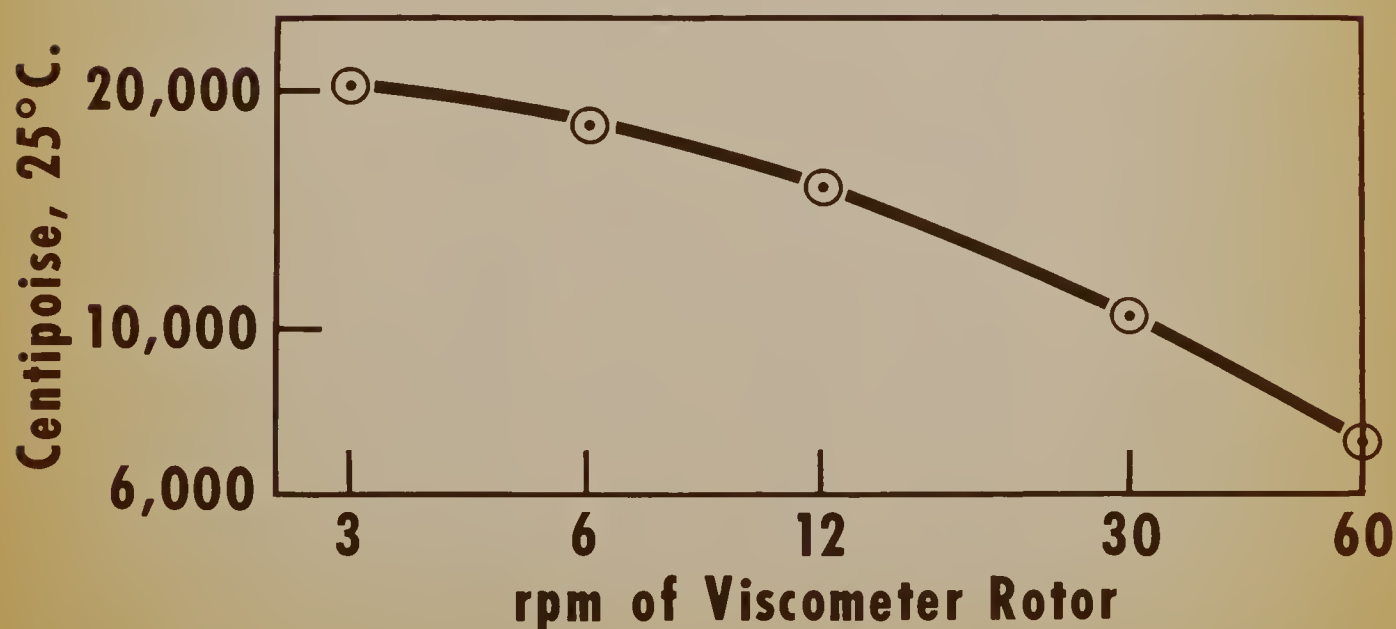


Fig. 2. Viscosity of 1.5% *Cassia marilandica* Gum vs. Rate of Shear



Grams per 1,000 seeds :	Species, NU No.	Mucilage, percent of air-dry seed N-free	CPR	Source
2.3	Astragalus hamosus, 41177	22.1	Good	Jones - Turkey
10.0	Cassia biflora, 38201	25.0	Fair to Good	J. Arguelles - Mexico
18.2	Cassia bonariensis, 42214	28.8	Good	F. A. Quinones - N. Mex. State Univ., Univ. Park, N. Mex.
26.2	Cassia corymbosa, 40756	18.4	Poor to Fair	Gentry - Miami, Florida
4.1	Cassia leptadenia, 42012	22.5	Fair to Good	J. Arguelles - Mexico
6.4	Cassia leptocarpa, 40718	30.2	Fair to Good	Gentry - Durango, Mexico
18.2	Cassia occidentalis, 41611	25.5	Fair to Good	W. T. Fike, Indonesia
17.0	Cassia occidentalis, 42215	32.4	Fair to Good	F. A. Quinones - N. Mex. State Univ., Univ. Park N. Mex.
3.0	Cassia sericea, 40130	22.3	Fair to Good	H. S. Gentry - Navojoa, Sonora, Mexico
12.0	Cassia sp., 40343	18.7		H. S. Gentry - Menores, Durango, Mexico
12.2	Cassia uniflora, 40726	28.8	Fair to Good	H. S. Gentry - Mexico
6.0	Crotalaria pumila, 41060	18.6	Fair	H. S. Gentry - Sonora, Mexico
15.0	Ipomoea sp., 40798	19.4	Poor to Fair	H. S. Gentry - Texas
13.8	Neptunia pubescens, 40896	20.1	Poor	Barclay, Perdue
4.8	Spinctospermum constrictum, 36392	23.6	Good to Excellent	H. S. Gentry - Mexico

Table 2. Paired feeding of Cassia marilandica fractions to rats

<u>Fraction</u>	<u>Total weight gain of three rats, g.</u>	
	<u>Experiment</u>	<u>Paired control</u>
Gum (No. 9)	+51	+53
Hull (No. 6)	-37	-14
Germ (No. 1), 47% protein	-35	-25

This gum, when fed to the extent of 10 percent of the diet for 16 days, supports growth as well as the pair-fed control.

Five percent germ from C. marilandica supports growth as effectively as soybean meal if the cassia germ is steamed (Table 3). Two other mucilage-bearing seeds show a preliminary test for toxicity (Table 3).

Table 3. Toxicity tests in rats: Five percent additives to the control diet for 29 days

<u>Additive, seed or fraction</u>	<u>Average weight</u> <u>g.</u>	<u>Remarks</u>
Soybean meal	131	
Cassia marilandica germ (No. 1)	113	
Cassia marilandica germ autoclaved	130	
Cassia bonariensis seed	-	all lost weight
Cassia occidentalis seed	-	all dead
Crotalaria intermedia germ (No. 1)	69	(for comparison)

#### IV. Status

Cassia marilandica gum, while not as effective a thickener as guar, should be tested as a paper size. Viscosity per se is not a measure of usefulness in this application. C. emarginata has previously been shown to have merit as a paper size. The protein of the seed is toxic, but the toxicity is apparently overcome by a simple heat treatment. Toxicity of the ~~raw~~ material probably would not constitute a serious agronomic problem, though more data may be required to settle the question. Further trial plantings should parallel the paper size investigation.

Cassia mimosoides (CPR Good) showed promise as a seed producer in 1962 plantings, and should be evaluated for toxicity before further work is done. (However, this has been recently identified as C. occidentalis which is toxic to rats; Note added January 1963).

Four other Cassia accessions need to be tested for toxicity. These are C. uniflora, biflora, leptocarpa, and leptadenia.

Sphinctospermum constrictum appears the most promising of the year's survey, and should be planted for preliminary agronomic evaluation.

Erlander - will be new introductions of Guar  
man from Okla in India.  
Dr Pully will bring in some.



## FIBER PLANTS FOR PULP

## I. Number of Samples Received, Analyzed and Evaluated:

	To Nov. 21, 1962	Dec. 1, 1961 to Nov. 1, 1962
Samples received	1111	167
Families	59	0 (new)
Genera	255	7 (new)
Species	492	34 (new)
Samples analyzed	739	65
Species	355	33 (24 new)
Promising species, basis alpha cellulose and MEA cellulose	35	4 (new)*
Other promising species		5 (new)*
Samples evaluated by small-scale pulping	131	12 (6 new)
Species represented	85	10 (4 new)
Individual cooks	234	20

\* As listed in following table.

New Promising Plant Species<sup>a/</sup> for Pulping (Dec. 1, 1961 to Nov. 1, 1962)

	Rating by analytical screening (Range 5-17; 5 is best)	Alpha- cellulose content %	Fiber length mm.	Fiber length- width ratio monocot > 90 dicot > 40	Preliminary ratings by small-scale pulping tests
<i>Triticum aestivum</i> (wheat straw) (for comparison)	9	34.5	0.89	60	9
<i>Urena lobata</i> <sup>b/</sup>	6 <sub>d/</sub>	34.4	1.06	56	-
<i>Wissadula cincta</i> <sup>c/</sup>	6 <sub>d/</sub>	34.5	0.65	54	-
<i>Horsfordia newbergi</i> <sup>c/</sup>	7 <sub>d/</sub>	34.0	0.74	54	-
<i>Hisbiscus lasiocarpus</i>	7 <sub>d/</sub>	33.2	0.95	44	-
<i>Arundinaria alpina</i> <sup>e/</sup>	5 <sub>d/</sub>	30.4	1.64	128	9
<i>Malachra alceaefolia</i> <sup>c/</sup>	6 <sub>d/</sub>	30.4	1.13	55	-
<i>Abutilon trisulcatum</i> <sup>c/</sup>	7 <sub>d/</sub>	32.1	0.80	78	-
<i>Anoda pentaschista</i> <sup>c/</sup>	7 <sub>d/</sub>	30.7	0.84	46	-
<i>Abutilon americanum</i> <sup>c/</sup>	7 <sub>d/</sub>	28.8	0.76	68	-

<sup>a/</sup> Analytical ratings potentially of 8 or less.

<sup>b/</sup> Source: Florida.

<sup>c/</sup> Source: Mexico.

<sup>d/</sup> Exclusive of botanical ratings which have not been available to IC.

<sup>e/</sup> Source: Ethiopia, Africa.

## II. General

Research on annual fibrous plants during this past year has continued along the lines discussed in the meeting of January 1962.

Analytical screening has been continued on botanically acceptable accessions, although their number has been diminishing during this past year.

Small-scale pulping was extended to include pulping at different levels of chemicals as means for detecting more favorable conditions for optimal strength development.

Emphasis in developmental pulping was shifted from papermaking pulps with the Hibiscus genus to boardmaking pulps by essentially mechanical techniques. Limitations on manpower prevented an effort being made along both avenues.

## III. Sorghums

Continued investigation of sorghums for papermaking has been in progress in a few foreign countries, principally France. We at NU have now completed preliminary evaluation of 128 sorghum samples, including 110 S. vulgare, 16 S. alnum, and 2 S. halepense (Johnson grass).

Very large variability was found between crop years when seed from the same PI accession was planted in the same location. Variability was also found between locations, with the poorer materials appearing to come from Texas whence most of the samples were derived. Nevertheless, taking the above factors into account, some lines appeared definitely preferable to others in pulping potential. These were S. alnum, PI 190579 and S. vulgare PI Nos. 229837, 34641, 88000, 88004, 177549, 179749, and 229847. For papermaking pulps the better sorghums provided yields equal to wheat straw and espartos but somewhat less than kenaf. The pulps were easily bleached and refined using minimal amounts of pulping chemical.

The bursting and tensile strengths of the pulps were inferior to kenaf and wheat straw, but comparable or superior to espartos. Zero span tensile data were comparable and even superior to kenaf and esparto, indicating possible improvements in the bursting and normal tensile strengths of the sorghums. Resistance to tear of the sorghum is inferior to kenaf and espartos. Opacity for the sorghums is intermediate to the kenaf and espartos. Bulk of sorghum sheets is comparable to kenaf and less than espartos. Folding endurance is comparable to kenaf and some espartos, the sorghum showing definite superiority over some espartos. Strengthwise the sorghums are superior to hardwood pulps. They do not approach the softwoods.

More plantings in Midwestern States of the particular accessions listed above seem desirable. Sorghum if used for pulp will probably find its preferred niche in fine papers or in board pulps. The latter possibility has not yet been evaluated at NU. Further developmental studies might profitably be made.



#### IV. Crotalarias

Thirty-four accessions of Crotalaria have been screened including representatives of the species juncea, incana, intermedia, mucronata, spectabilis, and striata. The strain of C. juncea introduced originally from Brazil was superior to other strains in that species. The genus is generally good. Pulp yields and bleachability were of the same order as those from kenaf, but the pulps had higher freeness, an important and necessary characteristic for today's high speed paper-making machines. This favorable freeness, and the satisfactory strength properties of crotalaria pulps, suggest additional developmental work when possible.

#### V. Developmental

##### Structural board pulps from kenaf

Considerable emphasis was placed on techniques for developing mechanical-type pulps from kenaf for use in structural boards. Industrial interest in kenaf and other dicots as raw materials is stimulated by the content of bast fiber contributing to the strength in derived products. Acceptance of such raw materials depends not only upon the physical properties that might be achieved, but also upon the competitive position with respect to wood. To avoid complications of stream pollution by spent pulping liquors, attention was directed toward mechanical-type pulps. Steam alone and limited amounts of neutral sulfite chemicals were used in preparing pulps. Pulps were prepared from both fractionated and unfractionated stalk material by appropriate milling and wet screening techniques. By controlled fiberizing of the classified fractions, products of reasonably acceptable fiber size and distribution as well as freeness have been obtained. These are to be used in board forming and evaluation studies. Pulp yields from cleaned and screened chopped kenaf have exceeded 90 percent. This figure is comparable to the accepted figure of 85 percent yield of groundwood from cleaned debarked wood. When yield of kenaf pulp is based on original stalks as harvested, the value drops to 83 percent. Debris (bark fragments and other fines) ranging from 5 to 12 percent of the original stalks as harvested account for the drop. Hardboards containing 10 percent phenolic resin have been prepared under exploratory conditions, but remain to be evaluated before systematic evaluation of pulps may be initiated. Two kenaf pulps approximating one processor's specifications have been furnished the company for evaluation.

##### Kenaf papers

A single experiment to produce bleached pulp and paper from bast fibers of mature kenaf was performed. Yields of unbleached and bleached pulps were 51.5 and 45.3 percent, respectively. The unbleached pulp exhibited exceptional resistance to tear with a maximum tear factor of 296 g./g.s.m. (a good grade of commercial soft-wool bleached kraft pulp had a maximum tear factor of 240 g./g.s.m.) and a zero span breaking length exceeding 16,000 meters. These results indicate the advantages strengthwise that might be achieved by use of

separated bast fiber. <sup>can</sup> Hurds which accumulate as a result of decortica-  
ting operations might be processed mechanically to a groundwood-type  
product for incorporation in furnishes for newsprint, toweling, book  
and magazine papers. The 3-stage bleaching technique used for the bast  
fiber pulp had a significant adverse effect on the strength properties.  
This might be corrected by application of more appropriate bleaching  
conditions that would be selected based on investigation of advanced  
techniques, such as those involving chlorine dioxide.

Bast fiber content of mature kenaf has ranged from about 18 to 29  
percent with an overall average of about 23 percent for 60 samples from  
various locations and growing conditions.

#### Industrial interests

As a result of the kenaf production test in southeastern Indiana with the  
Hillenbrand Industries, a variety of demands for raw material has been  
indicated by a small segment of the pulping industry. One firm, presently  
wishing to remain anonymous, has requested 10 tons of chopped material  
for large-scale pulping tests. This same organization had already  
evaluated kenaf and crotalaria to some extent through experimental pulps  
furnished by NU and also by their own small-scale pulping tests with  
chopped material from NU. Handsheets of newsprint-type papers were  
evaluated for strength and for printability in actual printing trials.  
This mill commented on the results as follows: "We were very encouraged  
with the printability testing results in furnishes containing the  
Crotalaria kraft. Although the strength is not equivalent to the long  
fiber substituted for, it was adequate. We feel that the long thin fiber  
in the Crotalaria is very desirable."

Ecusta Paper Operations, Olin, Pisgah Forest, North Carolina, has  
requested 500 pounds of the chopped Indiana kenaf for experimental  
studies. This company produces cigarette paper, carbonizing paper,  
condenser paper, bible paper and other fine grades mainly from tow  
fiber of the seed flax plant.

Peter J. Schweitzer Division, Kimberly-Clark Corporation, Lee,  
Massachusetts, producers of cigarette, condenser, carbonizing and  
bible papers from flax tow fiber, desires 10 pounds of the chopped  
kenaf for a pulping trial.

International Paper Company in its Southern Kraft Division at Mobile,  
Alabama, continues to evaluate experimental kenaf and crotalaria pulps  
from NU. Kraft wrapping and bag papers from long-fibered southern  
pines are an important segment of this company's production. As a  
result of an evaluation of an experimental crotalaria pulp, International  
reports in part as follows: "The low pulp viscosity indicates that some  
potential strength may have been lost in bleaching to 82 brightness  
compared to 75 for the bagasse pulp." The bagasse pulp was a commercial  
material used for comparison. Further, "Crotalaria juncea will apparently  
make a good short-fibered pulp which should be competitive in areas  
which are presently using annual fibers for pulp and paper manufacture."



Champion Papers continues to maintain high interest in kenaf, okra, sesbania and crotalaria as raw materials for papermaking. However, we are not aware of the present status of plans for establishment of the 100-ton-per-day mill with the "special circumstance" crop, crotalaria. According to an authoritative spokesman, the problem would be less difficult if the material didn't look so good.

J. E. Atchison, Parsons and Whittemore, Inc., New York City: "We are particularly interested in pulping work on crotalaria, kenaf and other materials of this type which might be combined with bagasse pulp mills in some of the areas we are working in."



REPORT OF CROP DEVELOPMENT RESEARCH

Chemurgic Crop Investigations  
New Crops Research Branch  
Crops Research Division

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Prepared by: J. R. Haun  
G. A. White  
December 19, 1962





Chemurgic Crop Investigations

35

This report summarizes the experimental plantings made in 1962 and earlier seasons that were not reported at the meeting last year. The order of presentation does not indicate priority or relative importance among the species discussed. For each potential new crop an effort was made to provide statements of relative promise based on the information available to date.

Progress has been made in the research on a few new crops (e.g., Crambe), but the work on other equally promising prospects has been limited by the relatively small staff that is currently available for crop development work. Research has continued on a small scale on bamboo, Dioscorea and Tephrosia. Reports on these crops are not included in the following discussions since they do not require joint crops-utilization attention at present. The research activity on bamboo is centered on the maintenance and observation of large-scale long-term plantings in South Carolina and Alabama. The activity on Dioscorea is reduced considerably and will consist of continued field experiments this year in select areas, using clones with high-sapogenin content. The effort on Tephrosia, in breeding of strains with improved rotenoid content, is located primarily at Mayaguez, Puerto Rico.

For comparison with the research programs on established crops and on chemical research, the following estimated professional man-year activity is assigned to the development of new chemurgic crops:

	<u>Professional man-years</u>		<u>Total</u>
	<u>NCRB</u>	<u>Cooperative Agreements in Universities</u>	
Crambe	1.0	0.5	1.5
Vernonia	0.25	0.2	0.45
Foeniculum	0.5	0.2	0.7
Limnanthes			
Lesquerella			
Preliminary Evaluations			
Dimorphotheca	1.0	0.01	1.01
Annual Pulp Fiber Crops	1.0	0.2	1.2
Bamboo	1.0	0.3	1.3
Dioscorea	0.75	0.15	0.9
Tephrosia	0.01	0.0	0.01
	<u>5.51</u>	<u>1.56</u>	<u>7.07</u>



Crambe

Iowa - Plantings were made at four locations, in twelve-inch rows at three levels of nitrogen fertility. Late season weeds were a problem. This type of weed growth would probably not reduce yields materially, but would make direct combining more difficult. Yields are not available yet. *700-800 lb/A in Southern field. 200-300 lb in N. low*

Missouri and Indiana - Results from 1962 plantings not available.

Minnesota - *N 10 613 lb/A Ind 300 lb 2000 lb/A* Three rates of seeding (11, 16 and 22 pounds per acre) in six-inch rows and eleven pounds per acre in twelve-inch rows were employed. Seedings were made with a conventional field grain drill and harvested with a combine. Yields varied from 1000 to 1150 pounds per acre.

Kansas - Yields ranged from 500 to 800 pounds per acre and some insect damage was noted.

Nebraska - In a date-of-planting study at Lincoln involving weekly plantings through April and May best yields (1400 to 1600 lbs per acre) were obtained from plantings made before May 2. Decreasing yields were obtained as the date of planting progressed to a low of 507 for the average of plots seeded May 23. The same study included three rates of nitrogen fertilization. This and other fertility studies suggest that a relatively low rate of nitrogen (25 to 75 lbs per acre) will be optimum for this area. In a spacing study increasing yields were associated with decreasing row widths in the four spacings used - 24, 18, 12 and 6-inch rows.

Wyoming - At Cheyenne the most comprehensive experiments were conducted in 1962. The mean yields from a spacing x irrigation x fertilization experiment were as follows:

<u>Cultural treatment</u>	<u>Yield in lbs/acre</u>
Irrigation	2254
Non-irrigated	620
Spacing 18"	1681
24"	1556
30"	1294
36"	1218
Fertilizer lbs 16-8-0 per acre	
None	1340
500	1558
1000	1414

Montana - Plantings were made at four locations - Moccasin (not successful) Bozeman (1106 lbs per acre), Kalispell (400 to 600 lbs per acre) and Huntley (510 lbs per acre). The latter three locations





were given 4, 2 and 12 inches of irrigation in addition to the rainfall of about 11, 4.6 and 10 inches respectively.

Washington - A two-acre planting at Pullman grew well and produced at the rate of about 800 lbs per acre without irrigation and essentially no rainfall. The soils of this area are noted for their productivity in the production of wheat.

Texas - An eight-acre planting at Weslaco yielded 450 lbs per acre. This planting was made after the record freeze in early January and harvested May 10.

A summarization of data from all locations is being prepared on cultural and environmental conditions including: soil type, drainage, frost dates, rainfall and temperature, irrigation, planting dates, fertilization, seeding rate, spacing, depth of planting, emergence date, equipment used in seeding and harvesting, plant size and yield, blooming and harvest dates, and insect and disease problems. Publication of this information by the New Crops Research Branch and cooperating groups will provide sufficient information for commercial production of Crambe in the more promising states. However, recommendations for widespread production cannot be made until sufficient commitments have been made for purchase of the seed by industries.



## Vernonia

Plantings were arranged at 10 different locations in 1962 with an aggregate area of approximately six acres. Although hand-harvested yields were promising on several locations, the total amount of seed actually collected for the Eastern Utilization Lab will be only a few hundred pounds. This was due to the difficulty of harvesting caused by shattering, and to irregular stands.

Nebraska - 1962 results - The following table indicates yields that were obtained in one of the experiments located at Lincoln.

Vernonia population - nitrogen test. Lincoln, Nebraska, 1962.

Row spacing	Seed yield (lbs/A) from			
	Nitrogen (lbs/acre)			
in.	0	25	50	mean
7	1219.9	1190.8	1202.4	1204.4
14	1361.9	1237.3	1133.7	1244.3
Mean	1290.9	1214.1	1168.1	

LSD (5%) N.S.

CV% 9.5%

These yields are slightly higher than in 1961. Again shattering was a problem.

Texas - 1962 results - Considerable difficulty was encountered in obtaining a stand of Vernonia this year. A one acre block on the A. & M. Plantation was planted four times between May 1 and June 18, 1962. The last planting on June 18 emerged to about 50 per cent stand. A one acre block on the Agronomy Farm was planted three times during the above period and no plants were obtained. Germination tests showed that the seed were viable (95 per cent germination). The only explanation that can be offered at this time is that the seed failed to germinate due to high soil temperatures.

North Carolina - 1962 report not available at this time.





Maryland - 1962 results - Seed germination was slow and irregular as in previous seasons. No explanation or cure for this problem has been discovered yet. A disease that developed during the season was identified as being caused by the cucumber mosaic virus. The disease was transmitted throughout the experimental plots by aphids which had overwintered on previously infected vegetable matter.

Indiana, Iowa, Kansas Minnesota and Missouri - 1961 results - No seed production was obtained at any station. The poor results were primarily due to poor germination. 1962 results - small quantities of seed are expected from most locations although yield data are not available yet.

Vernonia is still considered a good crop prospect. The problems of seed shattering, poor germination and disease control do not appear insoluble, provided that sufficient research effort can be applied.

Purdue - yields up to 1100 lbs

Gown. 140 - 270 lb/A

Kan. up to 657 lb/A

Mo. 300 lb/A



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Foeniculum

Fennel (Foeniculum vulgare) appears to be well adapted to the North Central states; however, its indeterminate flowering behavior and subsequent seed shattering causes less than maximum yields to be obtained.

Early seeding is necessary because fennel germinates slowly but growth is quite vigorous during the warm summer months. As is the case with most members of the Umbelliferae family, this plant is visited by many different insects some of which are harmful.

Only moderate intra-row weed competition occurred at Ames, Iowa, in a large plot (0.4 acre) planted in 1 and 2 foot rows with 4 levels of nitrogen. In addition, a few 1961 plots were retained and; although stands were not as thick as 1962 plantings, seed set was more uniform and advanced.

Status of 1962 Fennel plantings

Location	Experiments in progress		Retain for 1963	Yield prospect 1962	
	Spacings	N levels			
Indiana	x	x	*	fair to good	
Iowa			-		
Iowa State (484)	x	x	x	good	60 to 310
Regional Sta.				good	
Kansas	x		x	good	158 to 388
Minnesota			*	fair	
Missouri	x		x	fair to good	368 to 627

\* uncertain

Fennel seed analysis by the Southern Utilization Laboratory indicates that the oil content is affected by planting dates and, to a lesser degree, by plant spacing. Seed samples on this analysis were supplied from 1961 Nebraska plantings. These results, although based on limited determinations, suggest that further research of this nature is highly desirable.





# U. S. Seed and Oil Statistics of Certain Umbelliferous Plants

<u>Plant</u>	<u>Year</u>	<u>No. of farms</u>	<u>Acreage</u>	<u>Production in lbs.</u>	<u>Ave./acre in lbs.</u>
Dill (oil) *	1959	29	690	38,666	56
Dill (seed)	1951-1955	-	25	16,000	640
	1956	-	34	23,000	676
	1957	-	26	14,000	538
	1958	-	18	3,000	167
Fennel (seed)					
California	1949	1	4	1,000	250
California	1954	1	1	1,189	1,189
Connecticut	1954	4	9	2,350	261
New Jersey	1954	3	9	2,200	244
New York	1954	1	2	333	167
Celery (seed)	1958	-	36	36,000	1000
Parsley (seed)	1958	-	137	79,000	577
Parsnips (seed)	1958	-	58	53,000	914

\* States producing dill for oil in 1959 include Alabama, Idaho, New Jersey, and Oregon. Oregon produced 67% of the oil on 81% of the acreage involved. Highest yields per acre were obtained in Idaho (99.7 lbs/acre).

Fennel and dill seed can be successfully produced in Oregon and in the Imperial Valley of California, however, dill is easier to grow and slightly out-yields fennel. In these same areas, parsnip and celery yield approximately 1,000 pounds of seed per acre.

On the basis of yield reports and oil composition, it appears that other species, in addition to Foeniculum vulgare, could be included in further evaluation of petroselinic acid sources.



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 Estimated Petroselinic Acid Yield\*
 

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Plant	Seed	Seed oil		Petroselinic acid		Reference Number
	Lbs/acre**	% of oil	Lbs/acre	% of oil	Lbs/acre	
Dill	167-676	17.2	28.7-116.3	-	-	140
Fennel	167-1189	16.5	27.6-196.2	46.9	12.9-92.0	55
Celery	1000	17.0	170	33.7	57.3	26
Parsley	577	25.0	144.2	62.3	89.8	42
		19.0	109.6	45.0	49.3	132
Parsnips	914	17.3	158.1	-	-	19

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\* Oil and petroselinic acid percentages were extracted from "A Review on Petroselinic Acid and its Derivatives" by Lida L. Placek. Reference numbers used here refer to specific citations in this manuscript.

\*\* These yields are based on values from the preceding table.





Limnanthes

Maryland - Fall plantings at Glenn Dale developed well in the spring and bloomed profusely in early May. The plantings were irregular single row plots and yield estimations were not possible. The plants died immediately when hot days began. This performance confirms earlier observations that Limnanthes will not grow during hot summer months over most of the United States. It will be necessary to plant in the fall in areas where winter temperatures are not fatal, or possibly plant in early spring in areas where relatively cool temperatures prevail in the summer.

Twenty eight new accessions from the recent collecting trip have been planted as fall plantings in California, and, where sufficient seed was available in Georgia and Maryland (24 accessions). Selected accessions are scheduled for early spring planting in Minnesota, New York, Washington, and Wyoming.

The performance of these plantings in 1963 should provide sufficient information to determine whether Limnanthes can be developed into a crop. However, it should be emphasized that the small stature of this plant is such that mechanical harvesting may be difficult. Further, if the moisture and temperature requirements are too narrow it may be difficult to find sufficient areas for production. If unusual irrigation requirements are necessary, the costs of production may be excessive.



### Dimorphotheca

Not all locations reported 1961 results in time for inclusion in the January 1962 Progress Report. The following statements briefly summarize findings from these locations:

Very little, and in some cases no seed at all was obtained from plantings at certain locations in Oregon, Kentucky, Minnesota, Kansas, Missouri, Iowa, and Nebraska. Reasons for these failures were not always apparent but disease was the major causal factor.

A fertilizer experiment, which compared various combinations of nitrogen and phosphorus, was conducted at Corvallis, Oregon. It was planted April 15 and harvested with a vacuum cleaner on July 25 and August 30. Addition of fertilizer increased seed yields only slightly (from 499 to 575 pounds per acre). There was little evidence of disease or insect pests.

From past experience, it seemed that *Dimorphotheca* could best be grown in Texas as a winter annual. However, eight lines which had been selected for vigor of growth, were planted in a 2/3 acre plot on October 16, 1961; but all plants were killed in January when the temperature dropped to 12° F. No yield was obtained from a February planting because of poor stand.

Locations where plants survived long enough to produce appreciable amounts of seed were mainly in the summer-dry West. The more humid Midwest and South are less favorable because of foliage diseases. Premature plant mortality is also a problem in the West but the cause appears to be mainly root diseases.

For the past three years, very detailed and precise experimentation, largely basic in nature, has been conducted on *Dimorphotheca* and related plants at the Chico, California, station. A listing of this work is as follows:

1. *Dimorphotheca* location x species experiment
2. *Dimorphotheca* irrigation experiment
3. The germination of freshly harvested *dimorphotheca* seeds
4. The relationship of air temperatures to premature mortality of *Dimorphotheca* plants
5. Some characters associated with flower colors in *Dimorphotheca aurantiaca*
6. Effects of shading transplanted seedlings of three potential oil-seed species with wood shingles





7. Dimorphotheca broadcast seeding experiment
8. Seed yield of a dense population of Dimorphotheca sinuata
9. A comparison of furrow irrigation with sprinkler irrigation of Dimorphotheca sinuata
10. The effect of three levels of a complete fertilizer on the growth, survival, flowering, and seed yield of Dimorphotheca sinuata.
11. Description and evaluation of new species
12. Dimorphotheca selection plots of 1960
13. Harvesting of Osteospermum ecklonis seed before maturity to minimize losses from shattering.
14. Dimorphotheca plant spacing experiment of 1960
15. A comparison of plants grown from the two types of achenes of Dimorphotheca
16. The influence of light on the germination of seeds of D. sinuata and O. ecklonis
17. The effect of season of planting on the growth and development of Dimorphotheca

This research and work at other locations has provided valuable cultural information, but nonetheless tends to stress the only logically possible conclusion that certain existent problems must be overcome before this plant can be developed as a new crop. Several years would probably be required to acquire disease resistant and better seed retaining varieties because much selection and breeding would be necessary.

At the present time, there is one species of Dimorphotheca that offers a ray of hope - D. cuneata. This species has the advantages of high seed yield, short blooming period, good seed retention, disease resistance, and cold tolerance. Its principal disadvantages are poor seed form and slow growth rate, but the seed form is no worse than in D. sinuata and the slow growth rate is not much inferior to O. ecklonis. Provided there is sufficient interest in the seed oil from the chemical viewpoint, it is believed that this species merits further study.



Osteospermum ecklonis, although having good seed form, yield potential and disease resistance, tends to shatter badly and is not as cold tolerant as desired.

In view of the poor performance of most species under trial, it is recommended that work on Dimorphotheca be greatly reduced in favor of the more promising prospects such as Vernonia. Additional but limited evaluation of D. cuneata and Osteospermum species will be continued.





Lesquerella

Texas - About 0.04 of an acre of L. gracilis was planted on the Agronomy farm on November 21, 1961. The plants emerged December 7, and the first flowers were observed March 14, 1962. The seed crop was mature by May 22 and was harvested with a combine. Losses from shattering were estimated at 50 per cent. The yield was 135 pounds per acre. About 0.05 of an acre of L. grandiflora was also planted on November 21, 1961. The plants emerged on December 5 and first flowers were observed on March 6. The seed crop was mature on May 22 and was harvested with a combine. Again losses were estimated at 50 per cent and the yield (portion combined) was 298 pounds per acre. Seven other species were planted but poor or no stands were obtained and no seed were harvested.



## ANNUAL PULP FIBER CROPS

As potential sources of paper pulp, kenaf (Hibiscus cannabinus), Crotalaria juncea, and Sorghum alnum are considered promising for crop development. Dry matter yields in 1961 were, in general, superior to those of previous years. Although virus infection of Crotalaria has been reported, no serious disease or insect problems have evolved for these crops. Crotalaria can be substituted in nematode-infested areas for susceptible kenaf varieties.

Indiana - Purdue University - An early planting (April 26) of kenaf and Crotalaria was not as successful in controlling weeds as a planting seeded on May 14. A five year pulp crop improvement project has recently been set up at this institution.

Batesville - Five acres of kenaf were planted with a conventional grain drill on May 21, and plants emerged four to five days later. Weeds were well controlled by a 14-inch row spacing. There was no visible indication of a nitrogen response; however, final dry weight yields are necessary to assess any possible effects. The planting was quite uniform but an apparent moisture gradient resulted in a gradual shortening of plants toward the upper end of the five-acre tract. The remaining material, after pilot-plant quantities are supplied to two interested paper companies, will be utilized in a storage test and by the Utilization Laboratory.

North Carolina - Kenaf experiments in 1962 included spacing, varietal, and nitrogen trials. The spacing experiment consisted of four row-width distances of 7, 14, 21, and 28 inches and within row spacings of 3, 6, 9, and 12 plants per foot.

Nebraska - Supplementary water was added as needed to one of two identical kenaf nitrogen and spacing tests. Yield prospects are good.





## 1961 Yield Summary

Location	Row width in inches	Pounds dry matter per acre			
		Kenaf		Crotalaria juncea	
		Everglades 71	Everglades 41		
Nebraska *					
Lincoln	12	1376	-	2270	
Falls City	40	8340 **	-	-	
Humboldt	40	9120 **	-	-	
North Carolina					
Rocky Mount	12	12520	11980	-	
	24	8440	10200	-	
Plymouth	24	11700	12260	-	
Salisbury	24	11320	7840	-	
Oklahoma	40	8406	-	-	
Texas	12	11722	-	10275	

\* The combination of a cool wet spring and a hot dry summer period greatly restricted yields.

\*\* Air dry weights.



Preliminary Evaluations

The following table lists the species that were suggested for preliminary evaluation in 1962. Germination tests were made in April at Glenn Dale, Maryland, to determine which species would germinate under normal conditions anticipated for field plantings. The species that indicated sufficient viability were distributed as indicated. Further germination tests were run in October after the intervening storage period and in some cases special separations of good seed from trash, insect-injured seed or immature seed. The additional species showing viability will be distributed for planting through the regional programs in 1963.

PI Number	Name	Germination %		Regional			
		without	with	Plantings			
		treatment	treatment	made in 1962			
		April 1962	October 1962	NE	NC	S	W
279708	<i>Amblyolepis setigera</i> DC.	0	8				
279703	<i>Anchusa hybrida</i> Ten.	2	0				
279707	<i>Bifora americana</i> (DC.) Benth and Hook.	0	0				
279704	<i>Briza spicata</i> Sibth and Sm.	2	0				
279689	<i>Calendula officinalis</i> L.	8	-				x
279690	<i>Calendula officinalis</i> L.	13	-		x		x
279691	<i>Cassia alta</i>	0	20				
279709	<i>Cassia bonariensis</i> Colla	0	16				
279692	<i>Cassia leptadenia</i> Greenm.	0	8				
279693	<i>Cassia leptocarpa</i> Benth.	0	12				
194854	<i>Cassia mimosoides</i> L.	90	-		x	x	
279694	<i>Cassia occidentalis</i> L.	0	12				
279705	<i>Cichorium intybus</i> L.	1	56				
279710	<i>Comandra pallida</i> DC.	0	0				
279695	<i>Crotalaria longirostrata</i>	2	8				
279696	<i>Crotalaria</i> sp.	0	20				
279711	<i>Daucus pusillus</i> Michx.	0	0				
279712	<i>Dracopis amplexicaulis</i> (Vahl.) Cass.	31	-				x
279697	<i>Euphorbia parryi</i> Englm.	0	0				
279713	<i>Helichrysum bracteatum</i> Andr.	7	0				
279714	<i>Hibiscus syriacus</i> L.	1	0				
279698	<i>Ipomoea parasitica</i> Don.	96	-				x
279715	<i>Ipomoea</i> sp.	7	36				
279716	<i>Lappula redowskii</i> (Hornem.) Greene	0	0				
279717	<i>Lunaria annua</i> L.	7	32		x		
279718	<i>Lunaria annua</i> L.	8	0				
279719	<i>Marshallia caespitosa</i> Nutt. ex DC.	0	-				
279720	<i>Mercurialis annua</i> L.	3	40				
279721	<i>Penstemon spectabilis</i> Thurb.	0	0				
279699	<i>Polanisia viscosa</i> (L.) DC.	16	-				x
279722	<i>Salvia texana</i> (Sheele) Torrey	2	60				
279700	<i>Schkuhria wrightii</i> Gray	3	12				
279723	<i>Selenia grandis</i>	0	0				





PI Number	Name	Germination %		Regional			
		without	with	Plantings			
		treatment	treatment	made in 1962			
		April 1962	October 1962	NE	NC	S	W
279701	Sesbania sp.	0	0				
279706	Sideritis montana L.	46	-		x		
279724	Thalictrum dipterocarpum Franch.	0	12				
279725	Thalictrum polycarpum (Torr.) Wats.	0	0				
279726	Valeriana officinalis L.	11	32	x			x
279727	Veronica spicata L.	0	40				
279702	Zaluzania discoidea Gray	75	-			x	x

The following notes have been received to date on the performance of these accessions.

Georgia (and S-9 region) - PI 279699, Polanisia viscosa, made an excellent seed yield in the preliminary evaluation test at the regional station. Although this plant has an indeterminate fruiting habit, none of the capsules shattered. It appears that the seed from this plant could be easily combined after frost, or perhaps earlier if the plants were defoliated.

PI 204366, Cassia occidentalis, made satisfactory seed yields in preliminary trials at the regional station and at Clemson, South Carolina; Auburn, Alabama; and Fayetteville, Arkansas. This species also appears easy to combine. It has good seedling vigor and results from small plantings this year indicate that it can be grown with little or no difficulty in this region.

PI 279698, Ipomoea parasitica, made a good seed yield, but it would be difficult to grow and harvest on a large scale. It flowers over a long period, and a high percentage of seed is lost before the last seed is mature. Trellis support for the plants will probably be necessary for satisfactory yields.

Texas - Ipomea parasitica, Polanisia viscosa, and Zaluzania discoidea, were lost to weeds and grass due to prolonged wet soil.

North Carolina - An excellent seed yield of Cassia and lesser yields of Polanisia and Ipomoea were anticipated, however, final yield information has not been determined. Zaluzania did not grow well enough to establish a stand.

New York - PI 279717, Lunaria annua seed planted in the field gave only nine plants from 189 ft. of drilled row. These nine plants grew very slowly all summer, did not attain blooming stage and on October 20 were dug and placed in pots in the greenhouse.



PI 279726, Valeriana officinalis, grew very well, came into bloom August 18 and harvesting of the seed was started on September 15. The Valeriana plants have dichotomous branching with the flowers in cymose panicles. The seed is evasive and when it ripens has a pappus or tuft of feathery hairs on the smaller end. The seed itself is shaped like a bowling pin. As soon as the seed is ripe, the wind catches the tuft and blows it away. In order to collect seed, daily harvests would be necessary from September 15 to October 24. Its potential was not completely realized because many of the panicles had not ripened seed when frost came on October 24.

No further notes or yields have been received at this time.





# COMMERCIAL PRODUCTION AREAS FOR CRAMBE ABYSSINICA 1/

(In Selected States)

## The Problem

Agronomic and utilization research has shown that Crambe abyssinica is a potential commercial farm crop. The evaluation of this new crop must now be taken one step further: agronomic and economic factors must be considered to delineate commercial production areas.

## Criteria Used

Numerous factors influence the expected economic returns from present and new farm enterprises. Three important factors used to delineate commercial Crambe production areas were: (1) prices of input-outputs, (2) yields (production responses), and (3) weather (a risk and uncertainty factor). The first two factors were used to estimate the cost and returns for Crambe and alternative crops for crop reporting districts and land resource areas in Montana, North Dakota, South Dakota, Nebraska, Kansas, and Texas. The third factor was considered to minimize production risk arising from unexpected cold weather.

## Definitions

Prices: Prices of inputs and outputs were three year averages based on 1959-1961. The farmers' selling price for Crambe seed was assumed to be \$4.85 per hundredweight. 2/

Yields: The dryland and irrigated yields, in most cases, were ten year averages. Crambe yields were adapted from experimental data. 3/ These data were further adjusted to reflect yield differences among the crop reporting districts and land resource areas.

Cost of production: The cost of production includes those costs which could be allocated on an enterprise basis. Land rent, taxes, and depreciation on fences and farm buildings are not included. These costs are the same for each enterprise.

Break-even price: The break-even price is that price when multiplied by a specific yield equates returns and the cost of production.

Break-even yield: The break-even yield is the yield required to equal the cost of production, assuming a single price for the output.

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1/ By R. S. Corkern, Agricultural Economist, Market Potentials Branch, Marketing Economics Division, Economic Research Service.

2/ Based on prices of imported rapeseed oil and feed-grade meal.

3/ Based on yields obtained from experimental plantings in Wyoming of 2500 lbs. per acre on irrigated land and 800 lbs. per acre on non-irrigated land.

Weather: Number of freeze-free days was used as a weather variable and indicates the number of days between the last killing frost of spring and the first killing frost of fall.

### Alternative Crops

Table 1 shows expected levels of costs and returns from alternative crops at three yield levels for dryland farms in Montana. (Similar data, not shown here, were assembled for dryland and irrigated lands in North Dakota, South Dakota, Nebraska, Kansas, and Texas.) These data were used to delineate commercial production areas of the State where Crambe could be expected to yield a higher return than some present crops.

In several districts the alternative crop returns were less than their production costs. An example is rye in the Northwest crop reporting district of Montana. Crop situations like this invite replacement by Crambe, providing the economic returns from Crambe exceeds its production costs.

Crambe could also be substituted for some crops that are now returning a profit. An example is oats in the Northwest crop reporting district of Montana. For crop situations like this Crambe will be a more acceptable substitute if its returns equal or exceed the returns from the present crop. In most cases of substitution, the returns from Crambe must exceed the returns from present crops to compensate for the additional risk of growing a new crop.

### Comparative Returns

The returns from Crambe compare favorably, in specific areas, with returns from existing crops.

Tables 2 and 3 show several relationships that are of value in delineating Crambe production areas. These tables show for dryland and irrigated land:

- (1) A climate factor that is used to select areas that minimize production risk arising from unexpected freezes.
- (2) Alternative crops yielding a higher return than Crambe.
- (3) The alternative crops grown in a particular production area.
- (4) Crambe returns in excess of returns from alternative crops.
- (5) Those areas where the returns to alternative crops are less than costs of production.

As an illustration of the procedure for selecting land for commercial Crambe production, let us look at the Northeast crop reporting district of North Dakota (table 2). Crambe could be substituted for five crops now grown in this area. For which crops and in what order should Crambe be substituted? On the basis of the relationship in table 2, the following analysis may serve as a guide for making a decision:

- (1) Climate factor 1 indicates that the freeze-free period exceeds 120 days. The probability of a crop loss due to unexpected cold weather is minimized.
- (2) Crambe is not competitive with spring wheat since the returns from it exceed the returns from Crambe (designated by symbol "a").
- (3) Crambe returns exceed returns from corn, oats, spring barley, rye and flax (returns shown in the tables are the excess in returns for Crambe over each of these crops).
- (4) The returns from spring barley and rye are less than their costs of production (the excess return for Crambe is underlined).
- (5) Crambe should be substituted for rye first and spring barley second. (An acre of Crambe would return \$16.74 more than an acre of rye -- for barley it would increase returns by \$9.15 an acre.)
- (6) Crambe may be substituted for oats, flax and corn respectively. However, these crops give returns in excess of their costs of production. Farm operators would be more reluctant to shift from these crops to Crambe. But if a grower of oats, flax and corn made a substitution, he would replace them in this order.

The above analysis can be used to make other inter- and intrastate comparisons of returns from Crambe and other crops.

The above calculations do not take into account the variation of yields and prices for crops, including Crambe, that can occur. For example, in a given situation Crambe yield may be high while its alternative crop shows an average yield. In another situation, Crambe could be a low yielder when its alternative crop shows a high yield. Further comparisons between returns for average, high and low yield situations are necessary to allow consideration of these price and yield variations among the different crops.



Table 1.--Summary of production expenses, gross returns and returns above specified expenses per acre on dryland farms, Montana

Crop reporting district	Production expenses <sup>1/</sup>			Gross returns			Returns above expenses <sup>2/</sup>		
	Ave.	High	Low	Ave.	High	Low	Ave.	High	Low
	dol.	Yield dol.	dol.	dol.	Yield dol.	dol.	dol.	Yield dol.	dol.
Winter Wheat									
Northwest	14.62	14.87	14.47	45.63	54.08	40.56	31.01	39.21	26.09
North Central	16.08	16.38	15.68	40.56	50.70	27.04	24.48	34.32	11.36
Northeast	14.48	14.88	14.08	23.66	37.18	10.14	9.18	22.30	-4.74
Central	15.45	15.80	15.20	37.18	49.01	28.73	21.73	33.21	13.53
Southwest	14.42	14.72	14.22	38.87	49.01	32.11	24.45	34.29	17.89
South Central	15.74	16.09	15.14	35.49	47.32	27.04	19.75	31.23	11.90
Southeast	13.62	14.12	12.77	28.73	45.63	16.90	15.11	31.51	4.13
Spring Wheat									
Northwest	13.65	13.70	13.55	33.80	40.56	27.04	20.15	26.86	13.49
North Central	12.09	12.44	11.94	32.11	43.94	27.04	20.02	31.50	15.10
Northeast	12.27	12.52	12.02	23.66	32.11	16.90	11.39	19.59	4.88
Central	11.65	12.00	11.45	28.73	40.56	21.97	17.08	28.56	10.52
Southwest	14.12	14.47	13.87	33.80	45.63	25.35	19.68	31.16	11.48
South Central	12.06	12.51	11.81	27.04	42.25	18.59	14.98	29.74	6.78
Southeast	11.80	12.20	11.55	18.59	32.11	10.14	6.79	19.91	-1.41
Oats									
Northwest	13.52	13.73	13.31	20.65	24.78	16.52	7.13	11.05	3.21
North Central	11.54	11.81	11.42	18.29	23.60	15.93	6.75	11.79	4.51
Northeast	11.42	11.81	11.24	13.57	21.24	10.03	2.15	9.43	-1.21
Central	11.46	11.73	11.31	15.93	21.24	12.98	4.47	9.51	1.67
Southwest	13.26	13.53	13.02	21.24	26.55	16.52	7.98	13.02	3.50
South Central	11.49	11.82	11.16	16.52	23.01	10.03	5.03	11.19	-1.13
Southeast	11.12	11.30	10.85	12.39	15.93	7.08	1.27	4.63	-3.77
Barley									
Northwest	13.41	13.59	13.23	20.30	24.50	16.10	6.89	10.91	2.87
North Central	12.58	12.79	12.40	22.40	27.30	18.20	9.82	14.51	5.80
Northeast	12.41	12.62	12.29	13.30	18.20	10.50	.89	5.58	-1.79
Central	12.49	12.79	12.01	18.20	25.20	14.00	5.71	12.41	1.99
Southwest	13.41	13.68	13.20	20.30	26.60	15.40	6.89	12.92	2.20
South Central	11.60	11.99	11.30	17.50	26.60	10.50	5.90	14.61	-.80
Southeast	11.20	11.47	10.72	11.90	18.20	7.00	.70	6.73	-3.72
Rye									
Northwest	10.86	11.01	10.56	8.98	12.16	5.76	-1.88	1.15	-4.80
North Central	13.11	13.41	12.66	10.24	16.64	7.04	-2.87	3.23	-5.62
Northeast	11.80	11.95	11.53	7.04	10.24	4.48	-4.76	-1.71	-7.05
Central	11.14	11.38	10.75	7.68	12.80	4.48	-3.46	1.42	-6.27
Southwest	13.02	13.14	12.75	8.32	10.88	5.12	-4.70	-2.26	-7.63
South Central	11.17	11.38	10.87	8.32	12.80	6.40	-2.85	1.42	-4.47
Southeast	11.80	12.01	11.50	5.76	10.24	3.84	-6.04	-1.77	-7.66



Table 1.--Summary of production expenses, gross returns and returns above specified expenses per acre on dryland farms, Montana (cont'd)

Crop reporting district	Production expenses <u>1/</u>			Gross returns			Returns above expenses <u>2/</u>		
	Ave.	High	Low	Ave.	High	Low	Ave.	High	Low
		Yield			Yield			Yield	
	dol.	dol.	dol.	dol.	dol.	dol.	dol.	dol.	dol.
Corn									
Northwest	--	--	--	--	--	--	--	--	--
North Central	16.19	16.39	15.74	18.45	23.37	12.30	2.26	6.98	-3.44
Northeast	17.47	17.77	17.22	17.22	24.60	11.07	-.25	6.83	-6.15
Central	16.19	16.54	15.64	18.45	27.06	13.53	2.26	10.52	-2.11
Southwest	--	--	--	--	--	--	--	--	--
South Central	16.24	16.64	15.54	19.68	29.52	12.30	3.44	12.88	-3.24
Southeast	17.84	18.14	17.69	15.99	24.60	11.07	-1.85	6.46	-6.62
Flax									
Northwest	--	--	--	--	--	--	--	--	--
North Central	9.20	9.35	9.05	20.08	35.14	12.55	10.88	25.79	3.50
Northeast	8.98	9.18	8.68	15.06	25.10	10.04	6.08	15.92	1.36
Central	9.20	9.13	8.78	20.08	27.61	10.04	10.88	18.48	1.26
Southwest	--	--	--	--	--	--	--	--	--
South Central	9.10	9.20	9.00	15.06	20.08	10.04	5.96	10.88	1.04
Southeast	8.98	9.18	8.68	15.06	25.10	10.04	6.08	15.92	1.36
Mustard									
Northwest	--	--	--	--	--	--	--	--	--
North Central	6.89	7.06	6.75	24.18	32.06	17.68	17.29	25.00	10.93
Northeast	--	--	--	--	--	--	--	--	--
Central	--	--	--	--	--	--	--	--	--
Southwest	--	--	--	--	--	--	--	--	--
South Central	--	--	--	--	--	--	--	--	--
Southeast	--	--	--	--	--	--	--	--	--

1/ Land rent, taxes and depreciation on capital items such as farm buildings are not included in the production expenses.

2/ Returns to land, capital and management.

Table 2.--Production areas in which per acre returns from Crambe exceed the returns from alternative crops on dryland farms 1/

Alternative Crops

Crop reporting district	Climate factor <u>2/</u>	Spring Wheat dol.	Winter Wheat dol.	Corn dol.	Oats dol.	Spring Barley dol.	Winter Barley dol.	Rye dol.	Flax dol.	Mustard Seed dol.	Grain Sorghum dol.	Cotton dol.
<u>Montana</u>												
Northwest	3	a	a	b	11.81	12.05	b	20.82	b	b	b	b
North Central	2	a	a	15.08	10.95	7.52	b	<u>20.21</u>	6.46	.05	b	b
Northeast	2	a	a	6.04	3.64	4.90	b	<u>10.55</u>	a	b	b	b
Central	2	a	a	9.27	7.06	5.82	b	<u>14.99</u>	.65	b	b	b
Southwest	3	a	a	b	9.50	10.59	b	<u>22.18</u>	b	b	b	b
South Central	1	a	a	9.56	7.97	7.10	b	<u>15.88</u>	7.04	b	b	b
Southeast	1	a	a	<u>6.17</u>	3.05	3.62	b	<u>10.36</u>	a	b	b	b
<u>North Dakota</u>												
Northwest	2	a	a	8.61	10.21	4.66	b	10.21	8.61	b	b	b
North Central	2	2.48	b	<u>12.96</u>	<u>12.99</u>	5.73	b	<u>9.62</u>	8.04	b	b	b
Northeast	1	a	b	8.13	17.47	9.15	b	16.74	8.66	b	b	b
West Central	2	.15	a	8.65	11.81	<u>6.99</u>	b	<u>11.95</u>	10.70	b	b	b
Central	2	3.86	b	7.50	14.94	9.52	b	<u>12.88</u>	7.60	b	b	b
East Central	1	.43	b	10.15	19.07	<u>10.01</u>	b	<u>16.73</u>	8.91	b	b	b
Southwest	1	a	a	<u>11.85</u>	10.51	<u>6.12</u>	b	9.72	7.05	b	b	b
South Central	1	1.21	b	<u>10.13</u>	9.96	<u>6.32</u>	b	<u>10.69</u>	8.08	b	b	b
Southeast	1	1.62	b	11.60	15.02	<u>8.48</u>	b	<u>13.53</u>	11.16	b	b	b
<u>South Dakota</u>												
Northwest	1	.83	a	5.08	5.65	4.21	b	<u>9.48</u>	.84	b	b	b
North Central	1	1.84	a	<u>6.69</u>	5.28	5.71	b	8.65	5.12	b	b	b
Northeast	1	6.03	a	12.14	17.29	11.18	b	<u>15.97</u>	5.84	b	b	b
West Central	1	.06	a	<u>10.68</u>	5.00	3.69	b	<u>10.94</u>	2.30	b	b	b
Central	1	3.89	a	<u>8.08</u>	6.73	7.81	b	<u>12.00</u>	2.77	b	b	b
East Central	1	7.06	a	4.64	12.74	11.44	b	<u>18.49</u>	2.46	b	b	b
Southwest	1	7.12	a	11.90	12.11	9.50	b	<u>10.86</u>	7.10	b	b	b
South Central	1	4.03	a	10.86	7.28	5.72	b	10.09	b	b	b	b
Southeast	1	6.95	a	<u>4.81</u>	14.30	11.41	b	12.68	.57	b	b	b

Table 2.--Production areas in which per acre returns from Crambe exceed the returns from alternative crops on dryland farms 1/ (cont'd)

Crop reporting district	Climate factor 2/	Spring Wheat dol.	Winter Wheat dol.	Corn dol.	Oats dol.	Spring Barley dol.	Winter Barley dol.	Rye dol.	Flax dol.	Mustard Seed Dol.	Grain Sorghum dol.	Cotton dol.
<u>Nebraska</u>												
Northwest	1	a	a	9.84	4.85	3.66	b	b	b	b	<u>11.73</u>	b
North	1	a	a	.73	6.56	4.80	b	b	b	b	<u>8.29</u>	b
Northeast	1	6.49	a	2.05	<u>14.99</u>	14.99	b	b	b	b	<u>12.74</u>	b
Central	1	3.71	a	4.10	<u>7.95</u>	8.61	b	b	b	b	<u>6.57</u>	b
East	1	13.21	a	4.51	<u>13.80</u>	<u>14.62</u>	b	b	b	b	14.61	b
Southwest	1	1.61	a	7.61	4.68	2.52	b	b	b	b	<u>7.64</u>	b
South	1	5.55	a	8.29	<u>12.23</u>	<u>11.65</u>	b	b	b	b	<u>13.50</u>	b
Southeast	1	5.04	a	2.31	<u>9.47</u>	<u>10.19</u>	b	b	b	b	<u>10.00</u>	b
<u>Kansas</u>												
Northwest	1	a	a	b	11.88	8.11	8.48	b	b	b	12.36	b
North Central	1	a	a	11.16	11.17	<u>14.59</u>	<u>14.52</u>	b	b	b	<u>15.35</u>	b
Northeast	1	a	a	12.49	19.22	<u>20.99</u>	<u>20.48</u>	b	b	b	<u>22.88</u>	b
West Central	1	a	a	b	b	10.87	11.24	b	b	b	11.31	b
Central	1	a	a	6.89	6.93	<u>9.31</u>	<u>7.84</u>	b	b	b	<u>12.76</u>	b
East Central	1	a	a	11.01	13.74	<u>14.33</u>	<u>13.62</u>	b	b	b	<u>19.96</u>	b
Southwest	1	a	a	b	b	8.83	9.12	b	b	b	<u>10.15</u>	b
South Central	1	a	a	5.76	6.76	<u>8.10</u>	<u>6.71</u>	b	b	b	<u>12.15</u>	b
Southeast	1	a	a	8.72	12.47	b	<u>10.94</u>	b	b	b	<u>9.12</u>	b
<u>Texas 3/</u>												
High Plains	1	b	a	b	2.36	.71	b	b	b	b	<u>10.40</u>	a
Rolling Plains	1	b	a	1.59	4.29	8.00	b	b	b	b	<u>18.29</u>	a
Edwards Plateau 4/	1	b	4.56	b	2.45	1.46	b	b	b	b	<u>13.62</u>	1.44
Rio Grand Plain	1	b	.18	15.02	4.18	11.99	b	b	b	b	<u>12.41</u>	3.12
Central Basin	1	b	a	5.37	6.06	4.39	b	b	b	b	<u>10.88</u>	9.52
N. Central Prairies 1	1	b	a	11.52	4.97	b	b	b	b	b	14.73	a
W. Cross Timbers	1	b	a	12.13	6.67	10.24	b	b	b	b	<u>13.58</u>	a
Grand Prairie	1	b	.30	<u>2.43</u>	6.84	7.41	b	b	b	b	<u>12.16</u>	a
E. Cross Timbers	1	b	a	<u>12.13</u>	6.67	10.24	b	b	b	b	<u>13.58</u>	a



Table 2.--Production areas in which per acre returns from Crambe exceed the returns from alternative crops on dryland farms 1/ (cont'd)

Crop reporting district	Climate factor 2/	Spring Wheat dol.	Winter Wheat dol.	Corn dol.	Oats dol.	Spring Barley dol.	Winter Barley dol.	Rye dol.	Flax dol.	Mustard Seed dol.	Grain Sorghum dol.	Cotton dol.
Texas (cont'd)												
Blackland Prairies	1	b	a	6.61	13.01	10.59	b	b	b	b	14.22	a
Coast Prairie E. Texas	1	b	b	15.65	<u>19.96</u>	b	b	b	b	b	<u>20.39</u>	a
Timberland	1	b	1.59	16.10	<u>16.78</u>	9.99	b	b	b	b	<u>13.59</u>	a

1/ Alternative crop returns are for average yields and prices.

Crambe returns are for average yields and a price of \$4.85 per hundredweight. The figures in the cells indicate the amount Crambe returns exceed the returns from alternative crops. The underlined figures indicate those areas in which production costs exceeded the returns from the alternative crops.

2/ The climate factor represents the number of freeze-free days; 1 = 120 days or more, 2 = 100 to 119 days, and 3 = less than 99 days.

3/ Returns are shown for land resource areas.

4/ Eastern part of the Edwards Plateau.

a. Areas in which returns for alternative crops exceed the returns for Crambe.

b. Areas in which alternative crops are not generally grown.

Table 3.--Production areas in which per acre returns from Crambe exceed the returns from alternative crops on irrigated farms 1/

Crop reporting district	Climate factor <u>2/</u>	Alternative Crops							
		Spring Wheat	Winter Wheat	Corn	Oats	Barley	Grain Sorghum	Cotton	Flax
		dol.	dol.	dol.	dol.	dol.	dol.	dol.	dol.
<u>Montana</u>									
Northwest	3	42.28	43.54	49.86	70.86	70.61	b	b	b
North Central	2	46.94	37.36	60.54	69.68	69.42	b	b	51.63
Northeast	2	42.58	8.35	15.88	27.72	32.12	b	b	22.77
Central	2	28.86	24.75	32.50	52.16	53.11	b	b	37.13
Southwest	3	37.61	28.14	59.11	65.76	66.07	b	b	b
South Central	1	32.33	29.86	30.67	52.23	54.87	b	b	42.24
Southeast	1	11.22	8.90	23.00	27.92	32.13	b	b	7.79
<u>North Dakota</u>									
Northwest	2	29.42	27.66	b	62.02	54.48	b	b	b
North Central	2	30.72	31.73	64.59	62.48	53.05	b	b	b
Northeast	1	31.30	32.98	62.18	63.02	51.56	b	b	b
West Central	2	35.55	34.05	60.86	63.31	52.58	b	b	b
Central	2	33.22	32.49	63.88	62.46	53.47	b	b	b
East Central	1	2.01	32.98	62.18	63.02	51.56	b	b	b
Southwest	1	33.27	4.77	62.14	70.15	58.06	b	b	b
South Central	1	30.29	30.96	34.26	39.46	46.97	b	b	b
Southeast	1	30.72	32.98	60.94	63.02	51.12	b	b	b
<u>South Dakota</u>									
Northwest	1	51.02	51.74	54.55	63.99	62.41	b	b	b
North Central	1	46.97	43.08	57.36	63.10	59.90	b	b	b
Northeast	1	46.97	43.08	61.35	64.30	59.91	b	b	b
West Central	1	52.77	53.49	47.53	66.07	64.29	b	b	b
Central	1	46.97	43.08	50.98	63.10	59.90	b	b	b
East Central	1	46.97	43.08	50.98	64.30	59.91	b	b	b
Southwest	1	43.70	42.36	48.23	56.29	52.57	b	b	b
South Central	1	42.13	44.83	54.14	63.10	58.90	b	b	b
Southeast	1	44.40	44.22	54.14	61.46	59.80	b	b	b
<u>Nebraska</u>									
Northwest	1	20.89	b	23.45	38.81	36.79	38.72	b	b
North Central	1	1.12	b	24.29	46.96	46.25	39.03	b	b
Northeast	1	27.70	b	22.05	45.54	45.42	44.55	b	b
Central	1	7.45	b	24.27	52.68	50.78	43.02	b	b
East	1	26.71	b	22.92	45.22	44.43	42.71	b	b
Southwest	1	7.37	b	21.80	45.43	44.05	36.49	b	b
South	1	19.08	b	21.30	47.67	47.67	36.30	b	b
Southeast	1	27.66	b	18.07	45.66	54.38	41.91	b	b



Table 3.--Production areas in which per acre returns from Crambe exceed the returns from alternative crops on irrigated farms 1/ (cont'd)

Crop reporting district	Climate factor <u>2/</u>	<u>Alternative Crops</u>							
		Spring Wheat dol.	Winter Wheat dol.	Corn dol.	Oats dol.	Barley dol.	Grain Sorghum dol.	Cotton dol.	Flax dol.
<u>Kansas</u>									
Northwest	1	31.44	29.10	21.77	b	55.99	57.76	b	b
North Central	1	33.06	32.22	22.45	b	<u>57.29</u>	<u>56.83</u>	b	b
Northeast	1	37.55	35.28	23.64	b	<u>61.36</u>	<u>61.11</u>	b	b
West Central	1	31.44	29.11	20.77	b	<u>55.99</u>	<u>57.76</u>	b	b
Central	1	50.53	53.74	30.20	b	b	74.08	b	b
East Central	1	34.54	37.93	22.63	b	58.35	<u>57.39</u>	b	b
Southwest	1	51.23	50.64	28.97	b	b	<u>74.14</u>	b	b
South Central	1	50.23	54.83	31.29	b	b	75.17	b	b
Southeast	1	35.08	37.93	21.63	b	<u>59.24</u>	<u>57.39</u>	b	b
<u>Texas 3/</u>									
High Plains	1	b	53.55	b	b	62.14	<u>79.01</u>	a	b
Rolling Plains	1	b	51.70	b	62.06	b	<u>82.84</u>	a	b
North Central Prairie	1	b	b	b	60.22	b	<u>76.27</u>	a	b

1/ Alternative crop returns are for average yields and prices. Crambe returns are for average yields and a price of \$4.85 per hundredweight. The figures in the Cells indicate the amount Crambe returns exceed the returns from alternative crops. The underlined figures indicate those areas in which the production costs exceeded the returns from alternative crops.

2/ The climate factor represents the number of freeze-free days.

1 = 120 days or more, 2 = 100 to 119 days, and 3 = less than 99 days.

3/ Returns are shown for land resource areas.

a. Areas in which alternative crop returns exceed the returns for Crambe.

b. Areas in which alternative crops are generally not irrigated.

## NU PUBLICATIONS AND PRESENTATIONS

Additional to those listed in 1962 report

In Print

Fiber Dimensions of Nonwoody Plant Materials.

R. E. Perdue, Jr., and H. J. Nieschlag.

Tappi 44(11): 776-784. November 1961.

Alkaline Cleavage of a Homolog of Ricinoleic Acid.

M. O. Bagby.

Journal of Organic Chemistry 26(11): 4735-4737. November 1961.

Search for New Industrial Oils. VI. Seed Oils of the Genus Lesquerella.

K. L. Mikolajczak, F. R. Earle, and I. A. Wolff.

Journal of the American Oil Chemists' Society 39(2): 78-80.

February 1962.

Lunaria Seed Oil, A Rich Source of C<sub>24</sub> Fatty Acids.

T. L. Wilson, C. R. Smith, Jr., and I. A. Wolff.

Journal of the American Oil Chemists' Society 39(2): 104-105.

February 1962.

Amino Acid Composition of Lesquerella Seed Meals.

Roger Wayne Miller, C. H. VanEtten, and I. A. Wolff.

Journal of the American Oil Chemists' Society 39(2): 115-117.

February 1962.

New Sources of Seed Mucilages.

H. L. Tookey, R. L. Lohmar, I. A. Wolff, and Quentin Jones.

Journal of Agricultural and Food Chemistry 10(2): 131-133.

March-April 1962.

Purification of Erucic Acid by Low-Temperature Crystallization.

J. W. Hagemann, K. L. Mikolajczak, and I. A. Wolff.

Journal of the American Oil Chemists' Society 39(4): 196-197.

April 1962.

Isothiocyanates from Enzymatic Hydrolysis of Lesquerella Seed Meals.

M. E. Daxenbichler, C. H. VanEtten, H. Zobel, and I. A. Wolff.

Journal of the American Oil Chemists' Society 39(5): 244-245. May 1962.

The Search for New Crops.

I. A. Wolff and Q. Jones.

Yearbook of Agriculture 1962. U. S. Dept. Agr., pp. 473-476.

Fatty Acids, Fatty Alcohols, and Wax Esters from Limnanthes douglasii (Meadowfoam) Seed Oil.

T. K. Miwa and I. A. Wolff.

Journal of the American Oil Chemists' Society 39(7): 320-322.

July 1962.

New Industrial Crops--Some Economic Considerations.

Warren K. Trotter, Frederick J. Poats, and Ivan A. Wolff.

Agricultural Economic Report No. 10. 45 pp. June 1962.

Thalictrum polycarpum Fatty Acids--A New Class of Fatty Acids from Vegetable Seed Oils.

M. O. Bagby, C. R. Smith, Jr., K. L. Mikolajczak, and I. A. Wolff.

Biochemistry 1(4): 632-639. July-August 1962.

Densipolic Acid: A Unique Hydroxydienoid Acid from Lesquerella densipila Seed Oil.

C. R. Smith, Jr., T. L. Wilson, R. B. Bates, and C. R. Scholfield.

Journal of Organic Chemistry 27(9): 3112-3117. September 1962.

Amino Acid Composition of Seed Meals from Forty-One Species of Cruciferae.

R. W. Miller, C. H. VanEtten, Clara McGrew, I. A. Wolff, and Quentin Jones.

Journal of Agricultural and Food Chemistry 10(5): 426-430. September-October 1962.

Crambe - A Potential New Crop for Industrial and Feed Uses.

Crops Research Division, NU, in cooperation with Iowa, Montana, Nebraska and Texas Agricultural Experiment Stations.

ARS 34-42. 9 pp. September 1962.

Search for New Industrial Oils. VII.

F. R. Earle, I. A. Wolff, C. A. Glass, and Quentin Jones.

Journal of the American Oil Chemists' Society 39(9): 381-383.

September 1962.

A Search for New Fiber Crops. V. Pulping Studies on Kenaf.

T. F. Clark, G. H. Nelson, H. J. Nieschlag, and I. A. Wolff.

Tappi 45(10): 780-786. October 1962.

A Search for New Fiber Crops. Part VI. Kenaf and Wood Pulp Blends.

T. F. Clark and I. A. Wolff.

Tappi 45(10): 786-789. October 1962.



Submitted for Publication

Amino Acid Composition of Safflower Kernels, Kernel Protein, and Hulls and Solubility of Kernel Nitrogen.

C. H. VanEtten, J. J. Rackis, Roger W. Miller, and A. K. Smith.

Accepted for publication in Journal of Agricultural and Food Chemistry.

Analyses of Seed Samples from 113 Plant Families.

F. R. Earle and Quentin Jones.

Accepted for publication in Economic Botany.

Amino Acid Composition of Seeds from 200 Angiosperm Plant Species.

C. H. VanEtten, R. W. Miller, I. A. Wolff, and Quentin Jones.

Accepted for publication in Journal of Agricultural and Food Chemistry.

The Acetylenic Acid in Comandra pallida and Osyris alba Seed Oils.

K. L. Mikolajczak, F. R. Earle, and I. A. Wolff.

Submitted to Journal of the American Oil Chemists' Society.

Fatty Acid Composition of Maturing Vernonia anthelmintica (L.) Willd.

Seeds. Dihydroxyoleic Acid--A Possible Precursor of Vernolic Acid.

Thomas K. Miwa, Fontaine R. Earle, Glenda C. Miwa, and Ivan A. Wolff.

Submitted to Journal of the American Oil Chemists' Society.

Gums Separated from Crotalaria intermedia and Other Leguminous Seeds by Dry Milling.

H. L. Tookey, V. F. Pfeifer, and C. Martin.

Accepted for publication in Journal of Agricultural and Food Chemistry.

The Neuroactive Factor,  $\alpha$ - ~~$\gamma$~~ -Diaminobutyric Acid in Angiospermous Seeds.

C. H. VanEtten and R. W. Miller.

Submitted to Economic Botany.

Identification of Peaks in Gas-Liquid Chromatography.

Thomas K. Miwa.

Submitted to Journal of the American Oil Chemists' Society.

Presented at American Oil Chemists' Society Symposium, May 7-9, 1962, New Orleans, Louisiana.

Nuclear Magnetic Resonance for Determining Oil Content of Seeds.

T. F. Conway and F. R. Earle.

Submitted to Journal of the American Oil Chemists' Society.

Presented at American Oil Chemists' Society meeting, May 7-9, 1962, New Orleans, Louisiana; authorship: F. R. Earle and T. F. Conway.

Search for New Fiber Crops. Part VII.

Warren K. Trotter.

Submitted to Tappi.



Three New Oilseeds Rich in Cis-11-Eicosenoic Acid.

K. L. Mikolajczak, C. R. Smith, Jr., and I. A. Wolff.

Submitted to Journal of the American Oil Chemists' Society.

Presented at American Oil Chemists' Society meeting, October 1-3, 1962, Toronto, Ontario, Canada.

In Preparation

Fatty Acids, Fatty Alcohols, Wax Esters, and Methyl Esters from Crambe abyssinica and Lunaria annua Seed Oils.

Thomas K. Miwa and Ivan A. Wolff.

For publication in Journal of the American Oil Chemists' Society.

The Glycosidic Constituents of Ipomoea parasitica Seed.

C. R. Smith, Jr., L. H. Niece, H. F. Zobel, and I. A. Wolff.

For publication in Tetrahedron.

The Nature of the Pigments in the Seed of Dalea nutans.

C. H. VanEtten and R. V. Madrigal.

For publication in Economic Botany.

Oxazolidinethiones and Volatile Isothiocyanate in Enzyme Treated Seed Meals from 65 Species of Cruciferae.

M. E. Daxenbichler, C. H. VanEtten, F. S. Brown, I. A. Wolff, and Q. Jones.

For publication in Journal of Agricultural and Food Chemistry.

Presentations

A Chemist Looks at Plant Raw Materials.

Ivan A. Wolff.

Presented as Sigma Xi lecture, December 13, 1962, Brookings, South Dakota.

Long-Chain Wax Esters and Other Fatty Derivatives Prepared from Limnanthes douglasii, Crambe abyssinica, and Lunaria annua Seed Oils.

Thomas K. Miwa and Ivan A. Wolff.

Presented at American Oil Chemists' Society meeting, May 7-9, 1962, New Orleans, Louisiana.

Some Recently Discovered Fatty Acids of Unusual Structure.

C. R. Smith, Jr., and I. A. Wolff.

Presented at American Chemical Society meeting, September 9-14, 1962, at Atlantic City, New Jersey.

Patents

Method of Preparing a Drying Oil and Intermediates Therefor.

Cecil R. Smith, Jr., Curtis A. Glass, and Ivan A. Wolff.

U. S. Patent No. 3,027,388. March 27, 1962.

Method of Preparing 14-Hydroxy-cis-11-Eicosenoic Acid and Ester Derivatives Thereof.

Cecil R. Smith, Jr., Marvin O. Bagby, and Ivan A. Wolff.

U. S. Patent No. 3,057,893. October 9, 1962.

Process for Obtaining Undecanedioic and Nonanoic Acids in High Yields from Novel Plant Sources.

Kenneth L. Mikolajczak.

Patent application submitted.

Method of Preparing a Wax Ester Substitute for Jojoba Oil.

Thomas K. Miwa and Ivan A. Wolff.

Patent application submitted.





## APPENDIX

A Guide to Show Range of Seed Sizes for Comparison with  
Less Common Seeds

<u>Source</u>	Approximate Weight/1000 Seeds g.
Phaseolus vulgaris, kidney beans	300
Glycine max, soybean	200
Vigna sinensis, cowpea	130
Cyamopsis tetragonoloba, guar	35
Triticum aestivum, wheat	22
Crambe abyssinica, colewort	4.5
Vernonia anthelmintica, Indian ironweed	4.5
Trifolium pratense, red clover	2
Daucus carota, carrot (seed and pericarp)	1.5
Dimorphotheca sinuata, cape marigold	1
Trifolium repens, white clover	0.7



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